A Literature Review - Problem Definition Studies on Selected Toxic Chemicals.

Volume 3[illegible]

OCCUPATIONAL HEALTH AND SAFETY ASPECTS OF 2,4,6-TRINITROTOLUENE (TNT).

Final Report (11) Apr[illegible]78 (13) 111p.

by

(10) Samir Zakhari, Ph.D.
Jon E. Villaume, Ph.D.
[illegible]*tigator*
Paul N. Craig, Ph.D.

(16) 3E762720A835 (17) 00

(1) Final rept. rvsn '77-Apr '78,

Reviewed by

Frederick J. Goldstein, Ph.D.

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

(15) Contract No. DAMD 17-77-C-7020

Project Officer

Captain Ronald N. Shiotsuka, Medical Services Corps
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

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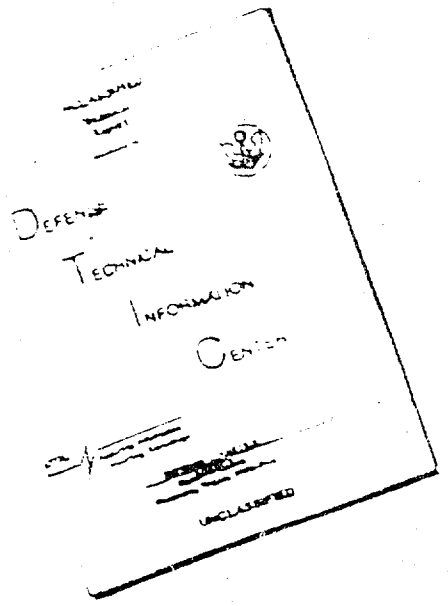

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) A Literature Review-Problem Definition Studies on Selected Toxic Chemicals. Volume 3 of 8 Occupational Health and Safety Aspects of 2,4,6-Trinitrotoluene (TNT)		5. TYPE OF REPORT & PERIOD COVERED Final Report March 1977 - April 1978 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Samir Zakhari, Ph.D. Principal Investigator: Jon E. Villaume, Ph.D. Paul N. Craig, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) DAMD-17-77-C-7020
9. PERFORMING ORGANIZATION NAME AND ADDRESS Science Information Services Organization The Franklin Institute Research Laboratories The Benjamin Franklin Parkway - Phila., Pa. 19103		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62720A 3E762720A835.00.030
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE April 1978 13. NUMBER OF PAGES 123
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; Distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Absorption	Biotransformation	Dietary factors
Analysis	Carcinogenicity	Distribution
Animal toxicity	Chemical properties	Epidemiology
Biodegradation	Degradation	Excretion

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A literature review (224 references) assessing occupational health and safety aspects of TNT (2,4,6-trinitrotoluene). Topics considered include human toxicity, epidemiology of TNT occupational exposures, industrial standards and practices delimited by the OSHA Act and U.S. Armed Forces recommendations on maximum allowable concentration of atmospheric TNT in the workplace. Laboratory animal morbidity and mortality studies, pharmacokinetics in mammals, microbial biodegradation, photodecomposition and other environmental aspects, and sampling and analysis in biologic media, air and water are reviewed.

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19. KEY WORDS (CON.)

Human toxicity	Physical properties	Teratogenicity
Industrial hygiene	Safety Aspects	TNT
Metabolism	Sampling	toxicity
Mutagenicity	Standards	2,4,6-Trinitrotoluene

20. ABSTRACT (CON.)

Recommendations for further research include determination of the carcinogenic, mutagenic and teratogenic potential of TNT, and establishment of a lower maximum allowable concentration in the workplace (from 1.5 mg/m^3 to 0.5 mg/m^3 atmospheric TNT) to reduce the occurrence of dose-related aplastic anemia, toxic hepatitis and jaundice, and other manifestations of intoxication from short- or long-term occupational exposure to TNT.

EXECUTIVE SUMMARY

This literature review (224 references) discusses various topics related to occupational health and safety of TNT, an explosive used in bombs, shells and grenades. It is a yellow powder and crystals at room temperature, and can be stored at room temperature for 20 years without decomposing.

Manufacture of TNT creates harmful fumes of TNT and other gases. Breathing the fumes can cause sore throat, sneezing or choking, and lung damage may occur, with recovery after several days. Workers who are exposed to TNT by breathing fumes or getting TNT on the skin may experience harmful effects including liver malfunction, decreased ability of the bone marrow to make blood cells, and death. TNT may also damage the heart, blood vessels, kidneys, pancreas, and possibly cause cataracts. Skin rash and redness may develop on the hands and forearms, collar area and ankles. Nausea and vomiting and stomach pains also are reported. The color of urine of persons with TNT poisoning is darker than normal, and may be red, but not bloody. Removal of the worker from all TNT fumes is important in treatment of TNT poisoning. Deaths are usually due to liver or blood disease. OSHA requires that workers in TNT plants are not exposed to more than 1.5 mg/m^3 of TNT in air, but disease still appears in some workers. The U.S. Army has lowered its acceptable TNT level to half of this amount, 0.5 mg/m^3 of TNT, in order to provide greater protection to exposed workers.

In animals, cats are more sensitive to TNT toxicity than rabbits, rats and monkeys. Liver disease and blood disease appear in animals exposed to TNT fumes. Dogs fed TNT showed no disease other than vomiting. Dogs which inhaled TNT fumes lost weight, and had vomiting and diarrhea and some blood problems but no liver disease. TNT cancer has not been reported in laboratory animals or humans exposed to TNT. But TNT did cause mutations in bacteria and chromosomal abnormalities in bone marrow cells of rats. The effect of TNT on reproduction is not known.

TNT can be absorbed into the body through skin, but much less enters the body by breathing fumes than through the skin. When fumes enter the mouth there is a bitter taste. The liver breaks down TNT which enters the body, and these breakdown products of TNT are found in urine but not in feces.

Bacteria . egrade TNT in waste water. Sunlight causes TNT to decompos in water, a gives the water a pink or red color. Organisms living in water can be harmed by TNT waste which is disposed in the water.

Persons working in TNT plants should not be exposed to TNT in air in concentrations above the OSHA limit. They should get medical examinations routinely and tests for blood and liver function, in order to detect signs of TNT disease early enough to cure the worker. Clean work clothes and showers after work may help avoid TNT skin contamination, and safety glasses, face shields and other protection are important.

The effects of TNT on reproduction and on unborn offspring, cancer from TNT, and eye cataract from TNT, are some topics which need to be studied.

ABSTRACT

A literature review (224 references) assessing occupational health and safety aspects of TNT (2,4,6-trinitrotoluene). Topics considered include human toxicity, epidemiology of TNT occupational exposures, industrial standards and practices delimited by the OSHA Act and U.S. Armed Forces recommendations on maximum allowable concentration of atmospheric TNT in the workplace. Laboratory animal morbidity and mortality studies, pharmacokinetics in mammals, microbial biodegradation, photodecomposition and other environmental aspects, and sampling and analysis in biologic media, air and water are reviewed. Recommendations for further research include determination of the carcinogenic, mutagenic and teratogenic potential of TNT, and establishment of a lower maximum allowable concentration in the workplace (from 1.5 mg/m^3 to 0.5 mg/m^3 atmospheric TNT) to reduce the occurrence of dose-related aplastic anemia, toxic hepatitis and jaundice, and other manifestations of intoxication from short- or long-term occupational exposure to TNT.

FOREWORD

The U.S. Army Medical Research and Development Command has the task of assessing occupational health and safety aspects of various chemicals to which Army as well as civilian personnel may be exposed. Accordingly, this Problem Definition Study (PDS) for TNT has been prepared as part of this research program under contract number DAMD-17-77C-7020, in order to provide the published data relating to occupational health and safety aspects of TNT. The subjects covered in this report include physical and chemical properties, methods of analysis, toxicological studies on humans and animals, metabolism, industrial hygiene and safety practices, among others. An appendix lists the sources examined to locate relevant information in the literature. Also included in the appendix is a list of various organizations contacted to obtain relevant information concerning TNT.

Problem Definition Study III is the third in a series of eight reports prepared under this contract.

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I. INTRODUCTION

The explosive 2,4,6-trinitrotoluene (TNT) was first prepared by Wilbrand in 1863; its production on an industrial scale in Germany started in 1891 (1). Ten years later, it began to find wide application as an explosive for shells, bombs and grenades, and gradually displaced picric acid. Millions of tons of TNT were produced during World Wars I and II and were extensively used as a bursting-charge explosive, usually mixed with oxygen-rich substances, as an ingredient of binary explosives, e.g., amatols of metalized explosives, e.g., Tritonal, and of commercial explosives, e.g., dynamites. Workers are, therefore, exposed to the toxic effects of TNT during manufacture, filling of bombs and shells and during washing of expired or unused bombs. Another source of exposure to TNT is during washing of the physical plant facilities.

TNT is the most widely used explosive because of its low melting point. It also gained great importance for military use because of its comparative safety in manufacture, transportation and storage, stability in contact with metals and nonhygroscopic nature (2, 3). TNT is also used as an intermediate in the synthesis of both dyes and photographic chemicals.

The information in this review is set forth in 10 chapters by categorizing the data into: physical and chemical properties; human toxicity and fatalities; toxicologic investigations in animals; carcinogenicity, mutagenicity and teratogenicity; relationship between dietary factors and TNT toxicity; absorption, distribution, biotransformation, excretion, and biodegradation; epidemiology; industrial health hazards, hygienic and safety measures and standards for TNT; and sampling and analysis of TNT. This section is followed by part B where the technical summary, concluding remarks and recommendations are discussed.

II. PHYSICAL AND CHEMICAL PROPERTIES

The physiological and eventually the toxicological actions of a given molecule are greatly affected by the physico-chemical properties which, in turn, are closely related to the chemical constitution of that particular substance. The very nature of the present compound, viz., trinitrotoluene, being extensively used as an explosive, necessitates a thorough description of its physico-chemical properties; this helps not only in understanding its hazardous effects on living creatures, but also throws more light on the ways of prevention and treatment of its toxicity. In order to meet the need for a comprehensive monograph and to bring it up-to-date with the ever-expanding literature, an exhaustive survey of articles dealing with TNT has been performed, of which references 1 to 18 were consulted for physico-chemical properties of TNT.

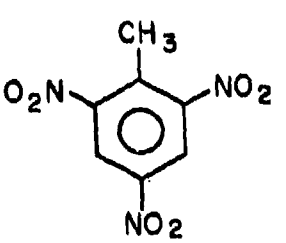
Toxic Substances List Number (1976): XU01750
Chemical Abstract Registry Number: 118-96-7
Wiswesser Line-Notation: T6N CN ENT J ANW CNW ENW
Chemical Name:-Toluene, 2,4,6-Trinitro (CA 8th CI)
-Benzene, 2-Methyl-1,3,5-Trinitro (CA 9th CI)

Synonyms (1):

2,4,6-Trinitrotoluene
TNT (The name used in this monograph).
Alpha-TNT
Sym-Trinitrotoluene
Trinitrotoluene
Sym-Trinitrotoluol
Tolite
Trotyl
Tritol
Triton
Trilite
TNT-Tolite (French)
2,4,6-Trinitrotolueen (Dutch)
2,4,6-Trinitrotoluol (German)
Trojnitrotoluen (Polish)

Molecular Formula: $C_7H_5N_3O_6$

Structural Formula:



C = 37.01%
H = 2.22%
N = 18.50%
O = 42.27%

Trinitrotoluene is solid at ambient temperature existing as yellow monoclinic needles or colorless orthohombic crystals (2). It also exists in the isomeric forms (2,10) presented in Figure II.1. Removal of these isomers in TNT manufacture involves treatment with aqueous sodium sulfite (sellite) in an alkaline medium. The nucleophilic attack of sulfite ion on the reactive meta positions results in the formation of water-soluble sodium sulfonates (11). The bulk of commercial TNT generally consists of α-TNT; various amounts of the above-mentioned isomers are also present. The composition of a military grade TNT is mentioned in Chapter X.

Another by-product, namely tetranitromethane, a skin and pulmonary irritant that is produced during the manufacture of TNT, may exist in the crude product to the extent of 0.12%; it was assumed that certain toxic effects observed in persons handling TNT should be credited to this contaminant (12).

The following are the properties of pure TNT.

Molecular weight: 227.13

Melting point: 80.9°C; on melting TNT expands in volume by approximately 12%.

Boiling point: 240°C (explodes)

Freezing point: 80.75 ± 0.05°C; this property is more dependable in identifying the purity of TNT than its melting point.

Refractive Index: TNT crystals have the following values for sodium light: $\alpha = 1.5430$; $\beta = 1.6742$; $\gamma = 1.7170$

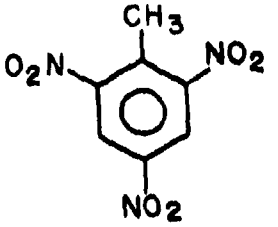
Specific gravity: 1.654

Viscosity: 9.5 cP at 100°C. Moore et al. (13) found that the temperature-dependence of viscosity of TNT fits by the method of least squares, the expression:

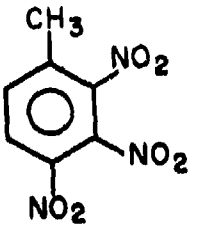
$$\log \eta = A + B/T + C/T^2$$

where A, B and C are constants and equal 2.78, -2.18×10^{-3} and 0.78×10^{-6}, respectively, and T equals 285°C.

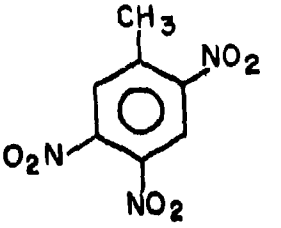
Solubility: The degree of solubility of TNT in various solvents (g/100g) is listed in the following table:



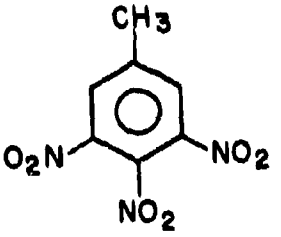
2,4,6-(α-)
Trinitrotoluene
melting point: 81°C



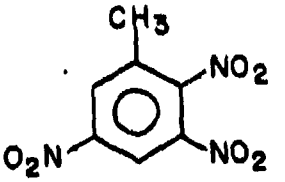
2,3,4-(β-)
Trinitrotoluene
112°C



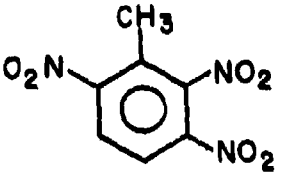
2,4,5-(γ-)
Trinitrotoluene
104°C



3,4,5-(δ-)
Trinitrotoluene
melting point: 137.5°C



2,3,5-(ε-)
Trinitrotoluene
97.2°C



2,3,6-(η-)
Trinitrotoluene
111°C

Figure II.1. STRUCTURES OF TNT ISOMERS

Solvent	Solubility in g/100 g of Solvent at Various Temperatures (°C)											
	0	20	25	30	40	50	55	60	70	75	80	100
Water	0.010	0.013			0.029			0.068				0.148
Ethyl alcohol	0.65	1.230	1.48	1.80		4.61				19.5		
Ethyl ether	1.730	3.290	3.80	4.56								
Acetone	57.00	109.00	132.00	156.00	228.00	346.00		600.00				
Carbon tetrachloride	0.20	0.65	0.82	1.01	1.75	3.23		6.90	17.34	24.35		
Chloroform	6.00	19.00	25.00	32.50	66.00	150.00		302.00				
Ethylene dichloride		18.70	22.00	29.00		97.00						
Trichloroethylene		3.04	3.50				60.00					
Chlorobenzene		33.90										
Carbon disulphide	0.14	0.48	0.63	0.85								
Methyl acetate		72.10	80.00	99.00		260.00						
Sulfuric acid		4.00										
Benzene	13.00	67.00	88.00	113.00	180.00	284.00		478.00			>2000	
Toluene	28.00	55.00	67.00	84.00	130.00	208.00		367.00			>1700	
Triacetin			37.7									
Butyl Carbitol Acetate		24.00										

Hygroscopicity: Practically non-hygroscopic (0.03% at 30°C and 90% relative humidity).

Volatility: Minimal at ordinary temperatures.

Vapor pressure:

mm Hg	at	°C
0.042		80
0.046		82
0.053		85
0.067		90
0.106		100
2.0		190
50.0		245 - 250

Flash point: Explodes at 240°C
E (activation energy) = 41.1 kcal/mole

Specific heat:

cal/(g)(°C)	0.309	0.328	0.353	0.374
temperature, °C	0	20	50	80

Heat of combustion: 3589.5 cal/g

Heat of fusion: 21.41 cal/g

Latent heat of sublimation: 28.3 kcal/mole (14), 24.7 kcal/mole (15).

Latent heat of volatilization: 22.7 kcal/mole (14).

Thermal conductivity: 0.00055 cal/(sec) (cm^2)(°C/cm) at 25°C.

Autoignition temperature: 275°C (confined); apparent activation energy(E)= 24,000 cal/mol (16).

Coefficient of linear expansion of cast TNT varies with crystal size, but for medium-sized crystals, it is 7.7 x 10^{-5} in/(in)(°C) in the range of -40 to 60°C.

Hardness of TNT crystals: 1.2 on the Moh scale.

Density: Cast TNT has a density of 1.55 to 1.56, but the crystals can be pressed to a density of 1.6 using a pressure of 50,000 psi.

Density of molten TNT: 1.465 g/cm^3.

Dipole moment: 1.37 Debye units.

Reactivity: Apart from its explosive property, TNT undergoes various chemical reactions with the following compounds:

1. Aldehydes: The methyl group in TNT is very reactive to aldehydic reagents. Thus, in the presence of the basic catalyst piperidine, TNT readily condenses with benzaldehyde to form 2,4,6-trinitrostilbene (3). With formaldehyde, TNT reacts to give 2,4,6-trinitrophenylethanol (2,4,6-trinitrophenethyl alcohol).

2. Alkalies: TNT reacts with alkalies, alkoxides and ammonia to form dangerously sensitive compounds, e.g., a solid potassium hydroxide and TNT mixture bursts into flame at 80°C (1).

3. Amines: TNT forms molecular complexes with aniline, toluidine, naphthylamine, and carbazole.

4. Acids: Chromic or nitric acid oxidizes TNT with the production of 2,4,6-trinitrobenzoic acid.

5. Sulfides and Sulfites: Sodium sulfide decomposes TNT completely with the formation of non-explosive products, a reaction which can be used to dispose of waste TNT. Sodium sulfite also decomposes TNT with the formation of a red, water-soluble product.

6. Photoreactivity:

Exposure of TNT, both as a solid and in solution, to strong sunlight or ultraviolet radiation, results in the formation of photodecomposition products. Initially, aqueous solutions of TNT turn pink in color; these gradually darken to yield, after a period of 4 to 6 hours, a cloudy, rusty-orange colored solution which is referred to as "pink water". Burlinson et al. (17) could identify only 8 photodecomposition products in pink water. These products which amounted to 20% by weight of the photolysis products were: 1,3,5-trinitrobenzene, 2,4,6-trinitrobenzaldehyde, 4,6-dinitroanthranil, 2,4,6-trinitrobenzonitrile and 4 isomers of dimethyltetranitroazoxybenzene, which have the structural formulae shown in Figure II.2.

Discoloration of TNT results in depression of the freezing point and an increase in its sensitivity to impact.

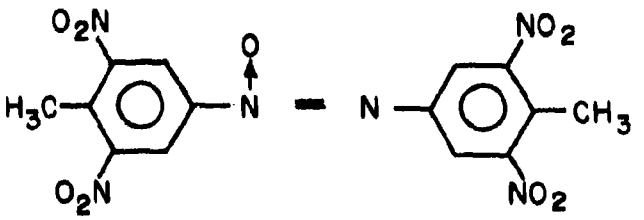
Stability: TNT is one of the most stable explosives. Vacuum-stability tests showed that at 150°C it underwent little, if any, decomposition in 40 hours. At 180°C, however, decomposition began and it exploded at about 310°C. After storage at ambient temperature for 20 years, at 65° C for one year, and at 75°C for six months, TNT has been found to be unchanged. Furthermore, molten TNT has been kept at 85°C for 4 weeks without deterioration. Molten TNT can be solidified and remelted as many as 50 times without decomposition. Nonetheless, storage of TNT at elevated temperatures may cause exudation of an oily eutectic mixture of impurities and TNT.

Sensitivity to Impact: Of all standard military explosives, with the exception of ammonium picrate, TNT is the least sensitive to impact and friction and has a very high explosion-temperature test value. However, the presence of gritty foreign materials (e.g. rust) renders TNT much more sensitive to impact. Furthermore, molten TNT is a dangerously sensitive material when confined, i.e. enclosed in a container, according to results of impact testing of solid or molten TNT, at temperatures ranging from -40 to 110°C (1).

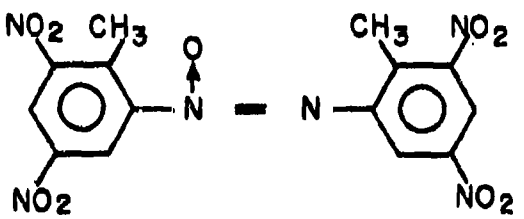
Crude TNT usually contains various amounts of impurities among which are β- and γ- isomers, traces of trinitrobenzene, trinitrobenzoic acid, dinitrotoluene and tetranitromethane.

Purification of TNT is accomplished by washing crude TNT with aqueous sodium sulfite solution (16%) which is strongly nucleophilic towards activated aromatic nitro group sites. Thus the 5- nitro of TNT, and the 3- nitro groups of β- and η-TNT are readily displaced by sulfite ion, which results in the formation of the water-soluble dinitrotoluenesulfonates.

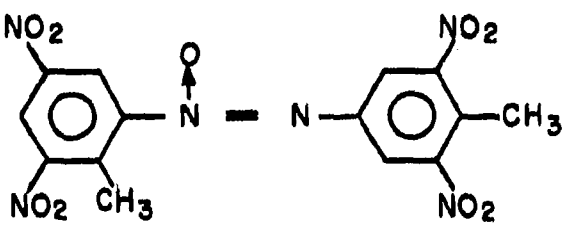
2,2',6,6'-tetranitro-4,4'-azoxytoluene



4,4',6,6'-tetranitro-2,2'-azoxytoluene



2',4-dimethyl-3,3'5,5'-tetranitro-ONN-azoxybenzene



2,4'-dimethyl-3,3',5,5'-tetranitro-ONN-azoxybenzene

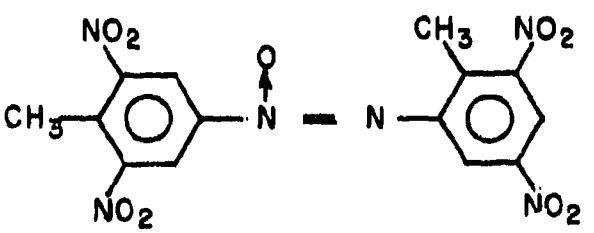


Figure II.2. STRUCTURAL FORMULAE OF SOME OF THE PHOTOLYSIS PRODUCTS OF TNT.

ref.: Burlinson et al. (17).

III. HUMAN TOXICITY AND FATALITIES

It was not until the manufacture of TNT began on a large scale in World War I that serious illness appeared among men and women employed in handling this compound. The danger of nitrous fumes that escape during the manufacture of TNT is by far less hazardous to health than TNT itself. While acknowledging the fact that TNT is the main source of toxicity for workers in ordnance plants, exposure to fumes of other substances during its manufacture may predispose and/or contribute to the toxicity of TNT; a comment on the effect of other fumes is, therefore, warranted.

Exposure of TNT workers to fumes in nitrating rooms, where toluene is mixed with sulphuric and nitric acids, causes clinical symptoms that are termed by Haythorn (19) "acute gassings". These symptoms are not due to TNT alone, but largely to nitric acid fumes, nitrous oxide, methane, hydrogen and chlorine gases. Individuals that are exposed to these gases become choked, develop cyanosis and sometimes lose consciousness. Severe bronchitis and edema of the lungs occasionally follows. Complete recovery after several days usually ensues, although bronchopneumonia and/or lobar pneumonia may occur. The usual respiratory flora, with a predominance of pneumococci, streptococci and staphylococci, were revealed on microscopic examination of sputa from bronchitis cases.

During World Wars I and II, several fatalities from TNT poisoning were reported (20-22) especially among workers who were exposed to either TNT fumes or dust, or those whose skin contacted this compound. Examination of more than 100 cases of TNT intoxication revealed that the greatest danger of exposure appears during the melting of TNT (22). However, like other aromatic nitro compounds, TNT may be produced and handled without injury to the exposed persons if rigid hygienic measures are enforced. In fact, this is the reason why there have been only occasional reports of deaths due to TNT poisoning since World War II.

In view of the fact that TNT, like other nitro compounds, exerts its toxic effects mainly on the liver and the hemopoietic system, a detailed description of its effects on these organs is mandatory. However, TNT is not without harmful effects on other organs, and, therefore the effects of TNT on individual organs, e.g., liver, heart, blood, blood vessels, eyes, kidneys and other tissues, are presented. This section is followed by a discussion of clinical manifestations and treatment of TNT intoxication; then a rapid survey of fatalities due to TNT is briefly mentioned.

1. TOXICITY TO VARIOUS ORGANS

To define the toxicity of TNT for each organ system and to ascertain the contribution of each of these systems to the general picture, the effect of TNT on each system will be briefly discussed.

A. Hepatotoxicity

Toxic hepatitis and aplastic anemia are the main causes of death in TNT poisoning. During World War I, 7,000 cases of TNT intoxication were reported to have occurred in a 20 month period in one plant in the United States, 103 of which ended fatally (1.5%) (23). In the period between 1916 and 1941, there

were 475 cases of toxic jaundice in British ammunition plants; of these 125 died (26.3%). During World War II, however, the number of fatalities significantly decreased; 22 fatalities occurred in the United States, of which 8 died of toxic hepatitis. Evans (24) also reported seven cases of TNT jaundice in workers in a TNT factory, of whom two ended fatally. Autopsy revealed acute yellow atrophy of the liver and hyperplasia of the bone marrow in one case and acute liver necrosis in the other.

Liver steatosis was reported by Hassman and Hassmanova (25) in a 55-year-old man who was in contact with TNT for 15 years. Liver damage was revealed by the icteric color of the conjunctiva and by the presence of bile pigments and urobilinogen in the urine. The excretion of these substances and TNT reduction products in urine imparts to the latter its dark coloration (26). Beside the increase in bilirubin in serum, patients subacutely or chronically intoxicated with TNT usually complain about pain in the right subcostal region and tenderness in the liver (See later for detailed clinical mainifestations). McGee et al. (27) considered cases of hepatitis positive if any two of the following could be detected:

1. Excess bile pigment in the urine
2. Impaired liver function test
3. Icterus index above six
4. Palpable tender liver

Symptoms of toxicity can be divided into two stages: the first stage is characterized by symptoms of catarrhal jaundice while the second is usually a rapidly fulminating acute atrophy of the liver (28).

As to the mechanism by which TNT brings about liver damage, Al'perin et al. (29) concluded that it is a direct effect on the liver cells. Jaundice apparently follows degeneration and the consequent obliteration of the bile capillaries, and is due to some form of toxemia which damages the liver cells producing subsequent autolysis and destruction (28).

Detection of Liver Damage

In an effort to prevent liver damage due to TNT, laboratory tests were performed as early as possible to diagnose any jeopardy to the liver. For this reason liver function tests were used in TNT plants. The thymol turbidity test as developed by MacLagen and modified by Daniel (23) was used to detect liver impairment. Though, in the strictest sense, this is not a liver function test, as Daniel reported "it furnished evidence of liver cell irritation" and "has proved most satisfactory". Present-day laboratory tests utilized in screening TNT workers for liver impairment include serum glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH). There are no tests reported, however, which determine TNT-specific hepatic impairment.

B. Hemotoxicity

Early findings of the effects of chronic TNT toxicity on blood created a great deal of controversy. Thus, while the presence of nucleated red cells, polychromatophila, anisocytosis and poikilocytosis were first mentioned in 1915 by Collins, as cited by Cone (30), as the major blood changes in individuals exposed to TNT,

Panton (31) two years later studied the blood of 50 female workers exposed to TNT (no concentration given) and noticed no significant changes in erythrocytes, hemoglobin or color index. Smith in 1918 (32) noticed no change in blood cells and hemoglobin of 25 men that were employed for 5 months or longer where TNT in its finely powdered form was used in the filling of shells. This is in agreement with Brill et al. (33) who reported a study of 17 workers in very intimate contact with TNT for 7 1/2 hours daily. Blood changes were monitored weekly for a consecutive 8-week period. Except for a transitory initial leukocytosis and moderate eosinophilia, no significant changes in the blood picture were found. In a review of TNT toxicity, Cone (28) reported blood changes brought about by exposure to TNT. He stated that about 60% of the workers have shown an early transitory leukocytosis and/or erythrocytosis, due probably to initial stimulation of the hematopoietic system. Determination of TNT concentration in air in that plant revealed a range of 0.5 to 2.0 mg/m^3 (30). Despite these findings, several authors indiciated the deleterious effects of TNT on blood and blood-forming organs. For instance, during World War II, 24 cases of aplastic anemia due to RNT were reported by Crawford (34) in the United Kingdom. Of these cases 9 persons recovered and 15 died. TNT has been reported as the cause of aplastic anemia, thrombocytopenia, and agranulocytosis (35). The effect of TNT on blood elements and blood-forming tissues will, therefore, be mentioned in detail.

1. Effect on red blood cells

Minot, in 1919, (36) showed evidence that with a very minimal amount of TNT entering the body, the formed elements of blood may be maintained at a slightly higher level than normal. This stage of stimulation in which all the marrow elements are kept above normal, may be masked by the destruction of the same when the amount of TNT is too great. Exposure to TNT may cause hemolysis of red blood cells, reduction in their oxygen-carrying capacity by the formation of methemoglobin (37) and nitric oxide hemoglobin (36), and permanent fixation of their pigment in the form of sulfhemoglobin. A study of the rate at which methemoglobin and sulphemoglobin are removed from blood of TNT workers showed that while methemoglobin is extracted by intracellular reducing enzymes within 2 to 5 days after removal of workers from contact with TNT, the latter substance persisted for 116 days, the lifetime of red blood cells (38). At the beginning of exposure, a slight degree of anemia may persist for several weeks, while the bone marrow maintains its capacity to produce blood cells.

Crawford (25) reported the blood picture of 27 cases of TNT intoxication. Red blood cell counts were further decreased in many cases due to bleeding from the gums and nose, retinal hemorrhage (3 cases), conjunctival hemorrhage (2 cases), and menorrhagia (2 cases). The red blood cell counts, as given by Crawford (1954) were as follows: (normal range/mm^3:men = 4.5 to 6 million; women = 4.3 to 5.5 million):

RBC Count/mm^3	No. of cases
630,000	1
730,000	1
1,000,000 or less	5
1,500,000 or less	11
3,000,000 or less	5

RBC Count/mm^3	No. of cases
3,380,000	1
2,790,000	1
3,504,000	1
no record	1

The hemoglobin content of the patients who died fell by 12% in one case, 14% in one, 17% in one, 20% in one, 21% in two and was present in varying quantity up to 50% in the remainder. However, the hemoglobin content of those who recovered was 17% (one case), 18% (one case), 20% (one case), 28% (one case) and varying up to 70% in the remaining eight. A decrease in hemoglobin content of red blood cells of more than 10% was considered a dangerous sign (34).

As to the size of red blood cells (taking the normal mean corpuscular diameter as from 6.6 to 7.7 μ), Crawford (19) reported upon 8 cases of macrocytic anemia (up to 8.4 μ) and 2 cases of megalocytic anemia (up to 10 μ).

The most noxious effects of TNT on blood, so far observed, are the hazardous depression of erythropoiesis in the bone marrow and the formation of methemoglobin. Brunetti and Grignani, in 1959 (39), reported upon 3 cases of hemolytic anemia caused by TNT in patients that were deficient in glucose-6-phosphate dehydrogenase (G6PD), and who showed decreased content and stability of reduced glutathione. Later, Djerassi and Vitany in 1975 (40) reported upon another 3 cases of hemolytic episodes in G6PD-deficient workers exposed to TNT. The most striking common feature in these cases is the onset of the disease which occurred within 2 to 4 days after the start of exposure. The authors recommend that investigation of G6PD activity of red blood cells in pre-emoloyment examination of prospective TNT workers should be considered.

Though hemolytic anemia was noticed in some G6PD-deficient persons, it is aplastic anemia that claimed the life of several TNT workers. Aplastic anemia is a rare disease that has a death rate of 3.5 to 5.4 per year per million of the population of the U. S. A. (41). However a number of cases of fatal aplastic anemia due to TNT intoxication has been reported (34,36,42-48). In certain susceptible persons the bone marrow may be damaged by TNT regardless of age, physical characteristics or previous employment. Most of these cases have had their onset during or immediately after a fairly long period of exposure to TNT. Furthermore, several cases of aplastic anemia were preceded by an episode of toxic jaundice or hepatitis. In one of these cases (45), a woman aged 35 showed pronounced jaundice after one month of exposure to TNT. Two months later she recovered completely from jaundice but nine weeks later, the patient showed clinical and laboratory evidence of aplastic anemia and died. Her liver showed typical nodular hyperplasia designated as subacute necrosis. However, Hayhoe in 1953, (46) reported upon a case of aplastic anemia occurring 8 years after TNT exposure. Since aplastic anemia could develop after a latent period from an attack of toxic jaundice, the case history reported by Hayhoe will be summarized. The patient, male, 45 years old, suffered from toxic jaundice two months after exposure to TNT in 1943. Icterus persisted for 2 weeks. After full recovery he was employed in another department of the same factory dealing with ammonium nitrate and aluminum dust, for a further two and a half years. Later, he worked

for a short period in a brewery and an ice cream factory before joining the water works where he spent 5 years before he was admitted to the hospital. Sternal marrow examination revealed aplasia of red cells, and granulocytes with 60% lymphocytes. Treatment with iron and vitamin B_{12} did not produce any hematological improvement, and transfusion of 2.8 liters of blood was given. The hemoglobin level fell steadily at a rate of 1 g% every 5 to 7 days. About 3 months after admission, the patient became comatose, developed respiratory failure, and died. Post-mortem examination revealed a large spreading hemorrhage extending throughout the left cerebellar hemisphere, submucosal hemorrhage throughout the small intestine and in the renal pelvis; the hemopoietic marrow of the femur was completely replaced by yellowish fat. Spleen and liver were congested and gave a positive prussian blue reaction.

4. Effect on white blood cells

Hamilton in 1946 (47) studied the blood picture of 105 individuals exposed to TNT and 142 others not exposed. Results showed that an early reaction to TNT intoxication is an increase in the large mononuclear leukocyte count, and usually this effect precedes any symptoms of illness from poisoning with this compound. This effect remains positive for two to three months and, therefore, can be used as a test for differential diagnosis of TNT poisoning even if Webster's test is negative (see Chapter X).

The white cell count in 13 fatal cases of TNT toxicity varied from 300 to 4,800 per mm^3, being 2,800 or less in eight cases. The count in 9 patients who recovered varied up to 5,200 per mm^3, the lowest record was 2,000 (one case) and 2,200 (two cases) (normal white cell counts range from 5 to 10 thousand). The differential white cell count was recorded in only 12 of these fatal cases and was as follows (34):

a. Polymorphonuclear leukocytes (normal = 40-60% of total white blood cell count)

% of white cells	number of cases
0-4	4 (agranulocytosis)
7	1
18-25	6
62	1 (massive hemorrhage)

In the 9 patients who recovered, the lowest neutrophil values varied between 28 and 84%.

b. Lymphocytes (normal range 20 to 40% of total white blood cell count)

The lymphocytes made up 31% of the white blood cells in one case (a woman with severe uterine bleeding), but averaged 78% in 9 of the fatal cases (61 to 92%). On the other hand, lymphocytes averaged 48% in patients who recovered.

c. Monocytes: (normal range 4 to 8%):

The percentage of monocytes in 10 of the fatal cases varied between 3 and 9% of the white blood cells; in aplastic anemic patients who recovered the range was 2 to 8%.

iii. Effect on blood platelets

In some cases of TNT intoxication, the number of platelets decreases. The low platelet count together with increased fragility of capillaries leads to toxic purpura (34). When anemia is coupled with thromobocytopenic purpura, the decline is lamentably swift. In the majority of fatal cases from toxic jaundice due to TNT toxicity, a purpuric eruption has also been noted at necropsy.

iv. Effect on bone marrow

The first reaction of the hemopoietic tissues to TNT poisoning is hyperplasia of the bone marrow; the shaft of the femur and the ribs are usually filled, to a surprising degree, with active red marrow (34). However, with further intoxication, the blood forming tissues begin to fail and the bone marrow becomes hypocellular. Similar findings were obtained by Germini et al. (1956, 48), who examined 13 cases of light chronic TNT intoxication. The bone marrow showed hyperplasia, myelocytes with toxic granulation, eosinophilia, giant platelets and increased reticular elements. Minot (36) ascribes the hyperplasia to the increased oxygen demand caused by the altered hemoglobin, as well as the products of blood destruction.

C. Cardiotoxicity

Soboleva in 1969 (49) studied 150 TNT workers, 134 of whom had been exposed for more than 5 years. Physical examinations revealed dullness of the first heart sounds in 96 patients, systolic murmur in 35, bradycardia (40-62 beats/min) in 63, systolic hypotension in 39, diastolic hypotension in 18, and simultaneous systolic and diastolic hypotension in 44. Further testing showed EKG abnormalities: left or right deviation of the electrical axes of the heart in 75; reduced atrial conduction in 34; reduced atrioventricular conduction in 3; reduced intraventricular conduction in 3; low-voltage QRS-complex and reduction of the height of T-waves in 97; $T_{V1} > T_{V6}$ syndrome in 27; prolongation of the isometric contraction phase of the left ventricle in 28; prolonged phase of asynchronous contraction in 5; and prolongation of systole in 34 cases. There was a dose dependent relationship between myocardial status and degree of occupational exposure. Myocardial dystrophy was diagnosed in 84 of 120 examinations of heart function. It should be noted that there were 38 men and 112 women in this study, and that 90% of them were under 40 years of age.

D. Vasotoxicity

The occurrence of cutaneous and mucosal hemorrhages as well as nasal bleeding in workers exposed to TNT, motivated Vychub (50) to study capillary permeability and strength in 82 workers suffering from TNT

intoxication due to exposure for 3 to 32 years. These workers included both men and women, 59 of whom were under 40 years of age. Vascular permeability was found normal only in 14 workers and was increased in the remaining 68, especially for proteins. Compared to a control group, patients suffering from chronic TNT intoxication showed increased vascular permeability in both directions, i.e., from the vessel into tissues and vice versa. These clinical findings were confirmed by experimental studies on dogs receiving an 8% TNT suspension in oil 3 times a week for 9 to 11 months. Histological and histochemical methods revealed morphological signs of an increased vascular permeability, e.g., perivascular edema, sclerosis and depletion of acid mucopolysaccharide in the vessels of various organs. The author claimed that changes in both capillary permeability and fragility can be used as a method of diagnosis of the early symptoms of toxicity.

E. Toxicity to Pancreas

The exocrine activity of the pancreas of 74 workers with clinical manifestations of chronic TNT intoxication was assessed by Kleiner in 1966 (51) who studied the activity of pancreatic enzymes (trypsin, amylase, lipase) and of the blood serum (total and atoxyl-resistant lipase, diastase), after administering a weak solution of hydrochloric acid or glucose to the workers. Functional disorders of the exocrine activity of the pancreas of the majority of patients, in the form of dyspancreatism of enzymes of the pancreatic juice and diastase deviation after double load with glucose, were observed. Five years later, the same author in 1971 published his results on 3 dogs chronically intoxicated with subcutaneous injections of small doses (0.1, 1.0 and 5.2 mg/kg) of TNT for 1.5 years. Functional disorders of the secretory activity of the pancreas and mild morphologic changes in the acinous apparatus were noticed (52).

F. Dermatotoxicity

According to Schwartz (53) dermatitis begins after 5 or more days of exposure to TNT and is often found at the points of friction such as the collar line, the belt line and the ankles. It usually starts between the fingers and the free edge of the palms with a superficial erythema which soon shows vesiculation (28). It is more serious during hot weather. The dorsa of the hands show edema and even the forearms up to the elbows may be affected. Seeberg (54) reported that skin lesions in explosive industry workmen healed when they ceased working with TNT. A detailed description of skin eruptions and suggested formulae for treatment were mentioned as early as 1916 by White (55).

Schwartz (56) stated that dermatitis is more prevalent where TNT is melted and poured into shells than in any other part of the operation.

Constitutional factors, personal hygiene and degree of skill of the worker all contribute to susceptibility to dermatitis. Historically, TNT workers believed that beer-drinkers, and possibly also tea-drinkers and neurotic women were more prone to dermatitis after occupational TNT exposure (18).

G. Oculotoxicity

Cataract formation, as a result of chronic trinitrotoluene toxicity, has been reported in several workers (57-61). Hassman and Juran reported cataracts in 26 cases out of 61 persons (average age 44.5 yrs) working with TNT for an average of 8.4 yrs (62, 63). In many individuals, cataracts occurred without other signs of TNT intoxication suggesting that the cataractogenic effect of

TNT may be due to direct effect on the lens (63, 64). The appearance of a cataract, according to Manoilova (65) is often the first and only sign of the presence of TNT in the organism, and this process does not always parallel clinico-hematological changes. Crystalline lens opacities may occur after vascular impairment to this region of the eye, which creates disturbances in local metabolic processes. The physical state of the TNT, workroom conditions, duration of occupational exposure and individual susceptibility to TNT all influence the degree of severity of cataracts (65). The development of a TNT cataract is gradual and usually takes several years, depending on the extent of exposure. Tyukina, in 1967 (66) reported that cataracts developed within 2 to 3 years in men, and within 6 years in women significantly exposed to TNT vapor and dust. Changes in the state of the crystalline lens occurred within 5 to 6 months, and four stages can be distinguished in the course of cataract development: appearance of peripheral dust-like opacities; distribution of opacities to cortical layer; increase in density of opacities; and finally, increase in size of opacities with the peripheral lens remaining transparent. These features were described as characteristic of TNT-induced cataracts, which could easily distinguish this cataract from lens opacities of different etiologies (66).

Development of cataracts was retarded and even a partial resolution of the opacities occurred when contact with TNT was stopped during early stages of cataract formation. Furthermore, local oxygen therapy, free base cysteine instillation and iontophoresis resulted in partial resolution of the cataract, even when work with TNT was continued. The only reported attempt to produce cataracts in animals was in 1964 when Pen'kov and Shershevskaya (67) introduced into the vitreous body of rabbits and guinea pigs, under local anesthesia, a granule of TNT. Widespread lesions of the crystalline lens and of the posterior part of the uveal tract occurred after one day. No visible changes were, however, noticed in the iris.

Munition workers usually develop irritation of the eye; toxic effects on the optic nerve are rare (68).

H. Teeth and Oral Cavity Toxicity

Workers in constant contact with TNT showed on medical examination characteristic carious and noncarious tooth injury, and peridontal and oral cavity mucous membrane disease (69).

I. Neurotoxicity

Medical examination of 19 workers handling TNT revealed a significant decrease of the chronaxy* of the flexors and extensors of the hand (70, 71). Clinical investigations on 140 patients carried out by Anatovskaya and Liman in 1972 (72) revealed the involvement of the nervous system in TNT intoxication. Neurasthenia, nystagmus, vagotonia, and hyperhidrosis were reported.

Whelan, in 1975 (73) reported a case of what is called "pelletol poisoning" which is intoxication occurring from the "nitrates" generated in the detonation of TNT and associated explosive products. The patient who was 60 years old worked as a "powder monkey" for 35 years and became ill while involved in doing some blasting off the coast of Alaska. Diencephalic seizures were manifested.

*chronaxy - the minimum time required for excitation of a structure by a constant electric current of twice the threshold voltage.

A recent study by Kaganov et al., in 1971 (74), showed that 20.8% of persons having long contact with TNT showed various disorders of the nervous system, chiefly in the motor and sensory neurons. Workers that had no signs of intoxication showed a definite prolongation of the optical chronaxy (2.5 times as compared with the control); of the vestibular chronaxy (1.5 times) and for the motor (3 times). Moreover, in 50% of persons examined a disorder in the thermoregulating reactions to heat and cold was observed.

J. Nephrotoxicity

Examination of 65 workers exposed to TNT versus 29 controls, revealed that in 19 cases out of those exposed to TNT, a significant rise in the glomerular filtration rate was observed as compared with the control group. Those handling TNT also demonstrated a partial retention of sodium. These findings were confirmed in experimental animals; thus, after prolonged exposure to TNT, dogs showed toxic renal dystrophy with marked anatomical changes in the glomeruli and tubules (75). In mild cases of TNT intoxication, urgency, frequent micturation and lumbar pain may be the only complaints (76).

Kennedy and Ingham in 1942 (77) discussed the incidence of porphyrinurea among TNT workers and observed that the number of positive cases decreased as the length of contact with TNT increased.

K. Biochemical Changes

1. Effect on plasma proteins and several liver-function tests

Stewart et at. (1945, 78) reported the early effects of exposure of 62 undergraduate students to TNT (age was not specified). These students were divided into 3 groups: group I (44 females) and group II (8 males) worked in the filling shops for an average of 33 days while group III (ten males) worked for an average of 18 days in the filling shops and 15 days in the melt houses. The average atmospheric concentration of TNT was 0.3 to 0.6 mg/m^3 in filling shops and 0.3 to 1.3 mg/m^3 in melt houses. The following biochemical findings were noted:

Biochemical test	Percent change		
	Group I (females)	Group II (males)	Group III (males)
1. Blood picture			
Hemoglobin	- 9.7	- 9.5	- 14.2
Erythrocytes	- 6.2	- 9.2	- 10.5
Hematocrit	- 4.9	- 8.5	- 10.0
Reticulocytes	+140.0	+250.0	+530.0
Leukocytes	- 22.0	- 2.0	+ 18.0
2. Plasma proteins			
Total plasma protein	-4.3	- 6.7	- 8.2
Plasma albumin	-4.2	- 8.1	- 8.1
Plasma globulin	-4.8	-13.6	-13.6
Plasma fibrinogen	-3.6	-21.6	+ 0.8
3. Liver function tests			
Total serum bilirubin	+36.0	+134.0	+179.0
Indirect bilirubin	+85.0	+ 40.0	+410.0
Direct bilirubin	0.0	+ 20.0	+100.0
Plasma phosphatase	+ 9.0	+ 21.0	+ 21.0
Hippuric acid test	- 1.0	- 6.0	- 2.0
Levulose index	+ 9.5	- 9.0	- 5.0

These changes can be summarized as follows:

a) A decrease in hemoglobin and circulating blood cells and an increase in the number of reticulocytes was noticed in 85% of cases.

b) A small but significant decrease in plasma proteins occurred in over 70% of the students after exposure to TNT.

c) A significant increase in the plasma bilirubin was observed in 20% of the cases.

d) No change in the levulose tolerance test; an increase in plasma phosphatase in 4 cases, and a decrease in hippuric acid excretion in 5 others.

e) Few students showed a fall in vitamin A content of the blood; however, no appreciable changes were noticed in vitamin C or carboxylase.

f) Men are more susceptible to the hemolytic effect of TNT than women.

ii. Effect on SGOT and SGPT

A group of 110 workers in a TNT plant were medically examined and no significant changes in SGOT, SGPT and SDH (sorbitol dehydrogenase) as compared with a control group were observed (79).

CLINICAL MANIFESTATIONS OF TNT TOXICITY

The main pathological manifestations of TNT toxicity are acute yellow atrophy of the liver, aplasia of bone marrow, petechial hemorrhage and nephritis. Clinical manifestations will be discussed under the following headings: A. signs and symptoms; B. findings on physical examination; and C. laboratory findings.

A. Signs and Symptoms

The symptoms of TNT toxicity may be mild and mainly due to irritation of skin and respiratory passages, or severe and due to absorption of a sufficient amount to produce toxic symptoms.

i. Mild irritative symptoms

a. Irritation of respiratory passages sometimes results in nasal discomfort, sneezing, epistaxis and coryza. This is usually associated with headache, perhaps due to nasal obstruction, sore throat and dry cough (26).

b. Dermatitis: Irritation of the skin by TNT usually leads to erythema and a multiplicity of polymorphic skin eruptions, usually on the parts of skin surfaces that are exposed to TNT, i.e., hands, forearms, neck, wrists and ankles. On the hands skin eruption is particularly evident between the fingers and on the thenar eminence, and usually starts as a papular eruption. On other affected parts, dermatitis takes the form of a coalescing papular eruption with areas of erythema. Desquamation follows, and may be severe, leading to complete exfoliation of the hands and feet (80).

c. Gastritis: Gastrointestinal disorders due to TNT are amongst the first signs of incipient intoxication and are usually manifested by the following symptoms: tightness in the chest and nausea, with or without vomiting; anorexia and constipation. The cardinal symptom is central epigastric pain that has no relation to food and is usually relieved by rest but not by vomiting. Slight epigastric tenderness, probably due to constant retching and vomiting is the only thing that might be revealed on medical examination of the abdomen (27).

ii. Severe toxic symptoms

Absorption of TNT dust or fumes through lungs or skin in sufficient amounts leads to precipitation of various gastrointestinal, circulatory and/or nervous involvements. These include:

a. Cyanosis: The most conspicuous sign of TNT toxicity is cyanosis and is due to the formation of methemoglobinemia. TNT workers show a greater or lesser degree of cyanosis which is usually manifest first in the lips, tongue, ears, fingertips and mucous membranes. Oxygen dearth is usually manifested by fatigue, lassitude, headache and dyspnea on exertion (6, 81).

b. Toxic jaundice: This constitutes the main symptom of toxicity that usually indicates severe liver damage which is responsible for 30% of the mortalities from TNT exposure. Jaundice, which usually starts in the conjunctivae and spreads rapidly to skin may or may not be preceded by an attack of cyanosis or toxic gastritis. Kleiner et al. in 1976 (82) after a study lasting more than 10 years, of 214 workers (of which 92% were women up to 40 years of age) with chronic TNT exposure, found that among the main clinical manifestations is toxic hepatitis with the syndrome of gall bladder dyskinesia.

c. Aplastic anemia: Chronic intoxication with TNT sometimes ends in aplastic anemia which is usually fatal (83). This is sometimes preceded by jaundice. Gregorson (84) attributed the pronounced fall in the percentage of hemoglobin and polymorphonuclear leukopenia to the absorption of bile constituents in the blood due to disturbances in bile circulation.

d. Cataract: Injury of the eyes in the form of a singular toxic cataract was observed by Zakharova and Manoilova in 45.3% of the 360 cases that manifested chronic TNT intoxication. It was claimed that sometimes cataract constitutes the first and only clinical sign of intoxication (85).

e. Menstrual disorders, dark scanty urine and dysuria: Disturbances in the menstrual cycle of young women at TNT plants are not uncommon. Hassman (86) in 1971, in a review article about TNT reported that both hypo- and hyper-menorrhea occur in women exposed to TNT.

f. Neurological manifestations: Ermakov et al. (87) after examining 574 patients exposed to TNT found that neurological disturbances were in the majority of cases functional and appear in the form of neurasthenia, nystagmus, irregularity of tendon reflexes and adiadochokinesia. Vegetative disorders were also observed and were chiefly caused by a rise in the tone of cholinergic innervation (vagotonia). This is manifested by hyperhidrosis of feet and hands, acrocyanosis, and lability of the pulse and arterial pressure. Bradycardia, positive clinostatic reflex,

poor pylomotor reflex and a change in diencephalic tests were also observed. Only in 2.2% of patients, did Ermakov and his co-workers find diffuse brain lesions.

B. Findings on Physical Examination

Patients usually complain of headache attacks in the forehead and temple area, dizziness, heart palpitation, shortness of breath during physical exercise, sweating, deterioration of memory and sleep disturbances. Clinical examination of workers handling TNT reveals the following:

i. Yellow discoloration of the skin, nails and hair (88). This is due to mere staining by TNT and should not be confused with the yellowish coloration due to jaundice. Parts of the skin that come in contact with TNT are hard and brittle, and those parts where skin is not stained are usually pale and sallow.

ii. More significant than the color of the skin is that of the mucosae. Usually lips show distinct bluish coloration, and a slight yellowing of the sclera is not uncommon. Cyanosis and dyspnea are common (89).

iii. Dermatitis, in the form of pin-head sized papules usually red in color on the extensor surface of the forearm is common. The lesion itches and worsens in hot and humid weather.

iv. Epigastric pain and tenderness and spasm in the epigastrium: this tenderness is most marked in patients who had clear signs and symptoms of intoxication and might be neurotic in nature.

v. The liver is usually enlarged in advanced cases and is distinctly palpable below the costal margin. This is definite evidence of liver degeneration and should be a signal for removal of the patient from work. In later cases of liver degeneration, jaundice is a prominent feature and might end fatally.

vi. Cardiac changes: Putnam and Herman (90), examining fifty TNT workers found that the lungs were normal, the knee-jerks and pupillary reactions were within normal limits, the pulse ranged from 62 to 104, respirations were from 12 to 28 per minute, blood pressure was apparently not affected and the temperatures were all between 97° and 99°F. However, this is in contradiction with Ermakov's et al. findings (87) who reported a decrease in the diastolic and mean arterial pressure in patients with chronic TNT intoxication. Furthermore, an increase in stroke and minute volumes (74%) was observed by the latter authors. The actual peripheral resistance decreased for all patients due to a decrease in the tone of small vessels, arterioles and precapillaries. Long-term exposure of workers to TNT resulted in instability of the autonomic vascular regulation and regional cerebral arterial hypotension in 62.5% of patients. The electrocardiogram reveals that many patients have bradycardia, a decrease in the amplitude of the QRS complex, and a flattening of the T wave. These changes might indicate myocardial dystrophy caused by the toxic effects of TNT. Hypotonia of the myocardium is due to methemoglobinemia and anemia. The increase in the vagal tone is reflected by the flattening of the T-wave, the increase in the P-Q interval, sinus bradycardia, and phasic sinus arrhythmia.

vii. CNS manifestations: Ermakov et al. (87) also reported a decrease in the amplitude of the brain's bioelectric activity of 78 of the hospitalized patients. The electroencephalographic changes consisted of a decrease in the amplitude of biopotentials, the appearance of slowed activity, and a poorer reaction towards stimuli. These changes are functional in nature and apparently caused by vascular disturbances in the deep regions of the brain. They claim that the lesions of the hypothalamus in chronic TNT exposure are due to the high degree of vascularization in that particular part of the central nervous system.

C. Laboratory Findings

Examination of urine, stools and especially blood gives direct evidence of the toxic effects of TNT.

i. Urine: The color of urine from patients suffering from TNT intoxication is usually darker than normal, ranging from abnormal amber to a fairly deep red. The specific gravity tends to be high and most important of all is that the Webster test is positive in most cases. Urine is usually acidic and is negative to Ehrlich's reagent.

ii. Stool: TNT could not be detected in stools and therefore, examination of stool might not furnish an important laboratory test.

iii. Blood: Ermakov et al. (87) examined 574 persons exposed to estimated concentrations of 1 mg/m^3 TNT for a period ranging from 6 to 25 years. They found normochromic anemia and leukopenia with relative lymphocytosis in 22% of patients; thrombocytopenia was also noted in 50% of the patients. Details of blood changes are mentioned above.

3. TREATMENT OF TNT TOXICITY

The main goals of treatment of TNT intoxication are to prevent further absorption and to enhance its excretion. Absorption is prevented by immediate removal from all contact with TNT. If TNT has been ingested, emesis or gastric lavage should be carried out; in case of skin exposure, changing of any contaminated clothing and thorough washing with soap and water is mandatory. Alkaline alcohol or an indicator soap was suggested (2) to confirm complete removal of TNT from the skin. A saline purge should be administered to help remove TNT (91). Furthermore, supportive measures for possible or actual liver damage should be included, e.g., high carbohydrate diet with plenty of fresh fruit and vegetables. Vitamin C and calcium in large doses are claimed to be of value in these cases (92). Gregorson in 1918 (84) indicated that tea and coffee can be given to patients ad libitum. Furthermore, milk, pudding, barley water, fish and rabbits can be provided to patients, but not in excess. He also mentioned that fatty food and saccharin should be avoided. Protective clothing was suggested by Shushkovskii (93) as prophylactic against TNT intoxication in mine workers. Absolute rest in bed and warmth are essential. The following measures are usually taken for treatment of dermatitis, aplastic anemia, jaundice and cyanosis:

A. Treatment of dermatitis: Immediate removal from contact with TNT is necessary, and generally is enough for recovery. However, some cases proceed to generalized eczema despite all treatment. Silver (18) gave the formula for "TNT paint" to be applied to the affected areas. TNT-induced dermatitis can be treated by the application of mild wet dressings such as boric acid solution, calamine lotion and solutions of aluminum acetate in the acute stages. After the acute symptoms subside the use of boric acid or zinc oxide ointment is recommended (53). Should lesions become infected, antibiotic ointments are recommended (94).

B. Treatment of Aplastic Anemia: Removal from contact with TNT is essential and careful cleansing of the skin to remove traces of the TNT is mandatory. Frequent transfusion of fresh blood is of great importance to support life. Other therapeutic measures include crude liver extract, yellow bone marrow extract, a high vitamin (C and B complex) and high caloric diet. However, in spite of intensive treatment, the prognosis is extremely grave and most cases have run a very short course (42). There is no certain favorable prognosis until the function of the bone marrow is actively restored. Until recovery of the bone marrow, extreme care should be exercised to guard against any infection, an attack of influenza, or even an injudicious dose of sulfonamide which may, at any moment, bring catastrophe (34).

C. Treatment of Jaundice: Jaundice is one very serious manifestation of poisoning and is life-threatening. Infusion of glucose and consumption of large amounts of carbohydrate orally are necessary to support the liver. It should be noticed that relapses are common and patients must be watched for an extended period of time.

D. Treatment of Cyanosis: Cyanosis may be treated with oxygen and carbon dioxide inhalation.

4. HUMAN FATALITIES

In the manufacture of explosives, accidental explosions are so great a threat that they overshadow the less spectacular but quite as real danger of TNT poisoning.

TNT was first synthesized by Wilbrand in 1863, and then gained great popularity as a blasting powder in peace-time, because of its high stability as well as its relative "low toxicity". This was the attitude towards TNT up to the onset of World War I when, because of reliance on the "safe" properties of the explosive, several poisonings and accidental explosions occurred. In the manufacture of TNT, there is always danger of nitrogen oxide fumes which gives rise to what is commonly called "fume sickness". However, there is less danger of intoxication from nitrogen oxide fumes than in the subsequent handling of TNT. It is in the melting, pouring, pressing, boring and planing of the charges for shells that the greatest number of cases occur. Both dust and fumes gain access to the body via the bronchial tree, and TNT can be also absorbed through the skin. Typical TNT intoxication takes place slowly because of the gradual degeneration of the parenchymatous organs. Eighty-three percent of cases of toxic jaundice have occurred between the 5th and 16th week of exposure to TNT (95). Death is mainly due to jaundice, aplastic anemia or both. The first fatality attributed to TNT intoxication was in 1915 (96), and by the end of that year, no less than

50 fatal jaundice cases resulted among TNT exposed workers (97).

In 1917, Hamilton (95) reported about 95 cases of toxic jaundice due to TNT exposure, of which 28 resulted in death, as declared by a member of the British government. He added that 703 more cases of TNT intoxication are in his list. Several fatalities have been reported since then (20, 98, 99). In the United States, within a period of about 7 months of World War I, about 17,000 cases of TNT intoxication were reported, of which 475 ended fatally.

According to Hegyeli (100), Telsinger reported about 24,000 cases of TNT intoxication in the U.S.A., of which 580 died during the period of 1914 to 1918. In the same period 384 cases were reported among munition workers in England, of which 90 ended fatally. In Germany, 1,000 toxic cases were reported and only 20 developed liver atrophy (98).

In 1927, Fortescue (101) reported TNT exposures among enlisted men that transferred 2,500 tons of cast TNT charges from their storage site. These charges had been in storage for a considerable period of time and a small quantity of a dark brown oily liquid, which consisted of TNT isomers with a small percentage of lower nitrotoluols, had separated out and covered the external surfaces of the containers. Toxicity was due to this exudate which volatilized rapidly in hot weather and was freely absorbed by skin and lungs.

Because of strict hygienic and precautionary measures, the number of fatalities during World War II has been decreased enormously. Thus in the period from June, 1941 to September, 1945, only 22 fatal cases have been reported (95). This number was reported from all Government-owned Ordnance explosives plants which produced 95% of all military explosives in the U.S.A. Eight of these 22 cases died of toxic hepatitis, 13 of aplastic anemia and one who partially recovered from hepatitis died from aplastic anemia or a combination of the two conditions. A detailed description of these 22 cases is given by McConnell and Flinn (97).

In 1942, seven fatal cases were reported (102). Patients were females ranging in age from 21 to 39 years; 4 of them died from toxic jaundice, 2 of aplastic anemia and one from acute toxic purpura.

After World War II, the number of cases of intoxication dropped, and were usually manifested by "sub-clinical events" (98).

Seland, in 1951 (103) reported upon a 70-year-old man who worked as a tunneller for 15 years. He developed aplastic anemia, which ended fatally in one year.

Jaundice and aplastic anemia are the most common causes of death from TNT intoxication. However, Crawford (34) examined 15 fatal cases due to TNT intoxication and reported the ultimate cause of death as follows:

Intractable recurring hemorrhages	5 cases
Thrombocytopenic purpura	3 cases
Agranulocytosis	3 cases
Anemia chiefly affecting the red cells	1 case
Brain hemorrhage	2 cases
Pulmonary embolism	1 case

Postmortem Findings

Microscopical examination revealed fatty changes in both liver and kidneys of 25 cases of fatal intoxication by TNT (104). Foulerton (105) examined the livers, kidneys and lungs of 3 women, 18 to 50 years old, that died from TNT intoxication and found the following pathological changes:

Liver: Advanced degeneration and disintegration of the parenchymatous cells together with advanced interlobular round-cell infiltration and fibrosis were observed. A large amount of fat was distributed in globules of various size partly in the interlobular fibrotic tissue and partly in or between the parenchymatous cells. Brown granular material was scattered throughout the liver.

Kidney: Sections in kidney showed accumulation of fat and cloudy degeneration of the epithelium of the convuluted tubules. Straight tubules in the medullary zone contained brownish material. No fat globules were observed in the glomeruli.

Lungs: Microscopic examination revealed marked cellular infiltration of the connective tissue stroma, but no marked exudation into the alveolar space was observed. Numerous fat granules as well as small masses of brownish material were scattered through the interalveolar tissues. Similar changes were found by others (106, 107).

SUSCEPTIBILITY TO TNT TOXICITY

Differences in susceptibility to TNT intoxication among males and females, blacks and caucasions or various age groups are disputed. Blacks were found to be less susceptible than whites in one study (28), and persons under age 20 were noted to be more susceptible than older age groups in a British report (95). Other studies found no differences in regards to age, sex or social susceptibility to TNT intoxication (178).

EFFECT OF EXPOSURE TO SUBTHRESHOLD CONCENTRATIONS OF TNT

The U.S. Occupational Health and Safety Administration Standard for TNT is 1.5 mg/m^3 in the U.S.A., and the maximum allowable concentration is 1 mg/m^3 in the USSR (86). Exposure to TNT in concentrations less than 1.5 mg/m^3 for long periods of time might have an adverse effect on the health of workers. Examination of 38 workers at TNT plants where the concentration of TNT varied from 0.1 to 1.2 mg/m^3 showed no sign of cataracts, nor any significant differences in hematological and liver function tests as compared to controls (108). However, in 1976, Morton et al. (109) reported the results of medical examination of 43 workers exposed to TNT for 8 hours/day. A significant increase in serum glutamic oxaloacetic transaminase and lactic dehydrogenase were found at TNT levels as low as 0.6 mg/m^3. The authors questioned the current value of 1.5 mg/m^3, suggesting a more adequate threshold limit value of 0.5 mg/m^3 of TNT in workroom air.

IV. TOXICOLOGIC INVESTIGATIONS IN ANIMALS

Experiments on various animals are the basis for most of our understanding of the toxicity of an unlimited number of compounds. In the case of TNT, experimental studies were stimulated by the large number of illnesses reported in munition workers during World Wars I and II, of which a considerable proportion ended fatally. Several species were used to study the toxic effects of TNT, and for convenience, this part will be discussed under the following headings:

1. Toxic dose levels
2. Species sensitivity and tolerance
3. Toxicologic differences between TNT isomers; toxicity of other substances generated during its manufacture
4. Acute toxicity
5. Chronic toxicity

1. TOXIC DOSE LEVELS

Table IV.1 summarizes various toxic doses that have been reported for TNT.

2. SPECIES SENSITIVITY AND TOLERANCE

It was found that, as in the case of other aromatic nitro compounds, the toxicity of TNT for animals varies with different species; this may be due, in part, to its different fate in the organism as suggested by Voegtlin et al. (110). Thus, cats were found to be very sensitive to TNT, whereas, monkeys, rabbits and rats showed little sensitivity (111). Values of 0.2 g/kg, 0.5 to 0.7 g/kg and 0.7 g/kg were reported to be lethal for cats, rabbits and rats, respectively. Furthermore, it was indicated by Voegtlin et al. (112) that there was a great difference in susceptibility of individual members of the same species to TNT. One dog receiving 10 mg/kg TNT showed more severe symptoms than another receiving 30 mg/kg. Animals with a slight grade of anemia or which have some infection are more susceptible to TNT toxicity. Dogs do not acquire tolerance to TNT, i.e., they do not become immune to the toxic actions of TNT (112).

3. TOXICOLOGICAL DIFFERENCES BETWEEN TNT ISOMERS: TOXICITY OF OTHER SUBSTANCES GENERATED DURING ITS MANUFACTURE

TNT, on prolonged storage may become covered with an oily layer of the β- and γ- isomeric trinitrotoluenes which does not affect "its efficacy as an explosive, but tends to increase its potential toxicity" (17). Although some authors reported that these isomers are of the same qualitative and quantitative toxicity (110,112) Panton and Bates (1921, 115) claimed that the α-compound is less toxic than the β-, and γ- seems to be better tolerated than in the case of α- in cats.

TNT preparation involves the stepwise nitration of toluene. Exposure to nitrous fumes (NO), which are generated during synthesis of TNT, is one of the first possible sources of toxicity. Panton and Bates, (113), therefore, exposed rabbits to NO fumes for various lengths of time. Their

TABLE IV.1. TNT TOXICITY SUMMARY

Animal Species	Strain or Type	Sex	Dose (mg/kg) and Dosage Form and Schedule	Route of Administration	Result	Duration of Experiment	Reference
Mouse		male	1,014 ± 52 4.12% solution	orally	LD_{50}	14 days	114
		female	1,009 ± 54 in peanut oil		LD_{50}	14 days	114
Rat	Wistar		150/day	orally	alive	120 days	115
			150 or 300 mg% of the diet (various diets)	orally	alive	90 days	116
			700	orally	LDLo		4
			>700	subcutaneously	lethal		114
		male	1,010 ± 41 4.12% solution	orally	LD_{50}	14 days	114
		female	820 ± 32 in peanut oil	orally	LD_{50}	14 days	114
Guinea pig			1 ml/kg/day for 10 days	subcutaneously	alive	9 to 12 months	117
			2 ml/kg/day	"	"	"	
			4 ml/kg/3 days	"	"	"	
			2 mg in aqueous suspension every 48 hours	orally	100% alive	60 days	118
			20 mg/week	orally	alive	35 days	119
			20 mg/week for 6 weeks	orally	70% died	6 weeks	119
Rabbit			500	orally	LDLo		4
			500 to 700	subcutaneously	lethal		114
			125 to 170 (in oil)	orally	alive		111
			170 (powder)	orally	alive		111
			125 to 176 (in oil)	subcutaneously	alive		111
			185 to 200 (in oil)	orally	lethal		111
			200 to 210	subcutaneously	lethal		111, 114
			250 to 260 (powder)	orally	lethal		111
			480	orally	lethal		114
Cat			1,850	orally	LDLo		4
Dog		female	5 to 100 mg in food/day 6 days a week	orally	100% alive	4 weeks	120
			25 to 50 mg/kg, 5 days/week	inhalation	67% alive	17 weeks	120
	Mongrel	female	50 mg/kg/day in food	orally	alive	12 weeks	120
	Beagie	both	0.02 to 1.0 mg/kg/day	orally	100% alive	13 weeks	223
Monkey	Rhesus	both	0.02 to 1.0 mg/kg/day	orally	100% alive	13 weeks	224

findings will be briefly discussed especially because they exclude NO as the causative agent of jaundice and anemia. In one experiment they exposed a rabbit (1.62 kg) to NO fumes for a total of 30 hours, 2 hours at a time; in the other experiment a rabbit (1.99 kg) was exposed for 2 hour periods nearly every day for 9 weeks, over a period of 3 months. Although the animal was cyanotic after each exposure and a positive Haldane's test for methemoglobinemia was found, five blood examinations revealed no appreciable changes in red-cell, white-cell, or differential counts, nor had bile been detected in the serum. Postmortem examination revealed no changes in liver, kidney, spleen or bone marrow. Lungs, however, were deeply congested and alveoli were filled with blood. Two rats were also treated in a similar manner; no blood changes were detected.

In the following section of this monograph, animal investigations on the toxicity of TNT, both acute and chronic, will be discussed. Data available on the toxicity and potential dangers of TNT show considerable diversity. The toxic effects of this compound have been discussed in detail in previous reviews (12, 68, 100, 111, 121).

In view of the fact that various animal species respond differently to TNT, it appears necessary to discuss the toxicity of TNT on different species, both in cases of acute and chronic exposure to this compound.

4. ACUTE TOXICITY

Experiments designed to determine mortality and other acute toxic effects of TNT are presented with respect to various species studied.

A. Mice

Administration of TNT (4.12% solution in peanut oil) orally to mice resulted in tonic convulsions that lasted for about 2 hours. Values of 1,014 ± 52 and 1,009 ± 54 mg/kg were reported for LD_{50} in male and female animals, respectively (114).

B. Rats

The acute oral toxic doses of TNT (4.12% solution in peanut oil) that killed 50% of male and female rats were 1,010 ± 41 and 820 ± 32 mg/kg, respectively. Animals underwent symmetrical coordinated convulsions within 5 to 15 minutes which lasted for 1 to 2 hours after administration. Animals that survived respiratory inhibition with associated convulsions usually appeared cyanotic and ataxic, and completely recovered within 24 to 48 hours. A bright red pigment appeared in the urine of rats within 10 to 20 minutes after administration (114).

Somers and West, in 1944 (122) reported the effects of administering TNT on urinary porphyrin output to male albino rats, maintained on a daily fixed weight of porphyrin-free diet. A suspension of TNT in 2% acacia mucilage in tap water was given every 2 days for 18 days. They found that TNT absorption took place more rapidly in the gastrointestinal tract than by subcutaneous injection. Doses higher than 400 mg/kg substantially increased the porphyrin excretion, as shown in the following table:

TABLE IV.2. PORPHYRIN EXCRETION IN RATS TREATED WITH TNT

Dose of TNT mg/kg	Route of Administration	Mean total urinary porphyrin (mg/100g/2 days) before	after	
Control	Subcutaneous	6.1	6.9	
200	Subcutaneous	4.6	5.5	
400	"	5.8	11.3	after sixth injection
600	"	4.0	11.8	" fourth "
Control	Stomach tube	no change		
200	Stomach tube	no change		
400	"	1.9	6.3	after second injection
600	"	5.4	15.5	" " "

Himsworth and Glynn (115), studied the acute effects of giving white Wistar rats 0.5 to 1.0 g/kg of TNT dissolved in arachis oil, subcutaneously. A few minutes after injection, rats passed urine which was bright red in color, and within an hour rats appeared gravely ill. After 48 hours, a proportion of the animals laid limply in the cage breathing slowly and weakly and then "quietly" died. Autopsy showed that the liver was shrunken and dark in color. Microscopic examination of the liver showed no evidence of fatty infiltration or necrosis.

Pidemsky et al. (123) found that the progressive inhibition of phagocytosis induced by TNT in rats can be prevented by the administration of niacin.

C. Guinea Pigs

TNT powder was given orally to guinea pigs in a dose of 0.2 to 0.5 g daily or every other day for 3 to 8 days, with a total dose of 0.6 to 2.0 g. Animals developed severe anemia mostly of the hyperchromic type, with an increased reticulocyte count, considerable leukocytosis and reduced platelet count (124).

In order to induce experimental TNT poisoning, Haythorn (19) administered TNT to guinea pigs and rabbits orally, subcutaneously (in oil), by intravenous (in serum) injection or by percutaneous application in lanolin. Neither the dose, the dosage schedule nor the duration of experiments were mentioned. However, after 2 to 3 days animals excreted brownish liquid stools, and moderate anemia was shown in 4 to 8 days. Cyanosis, myocardial depression and hypothermia were also noticed.

A 4.12% solution of TNT in peanut oil was topically applied to 10 guinea pigs in dermal sensitizing experiments according to the Magnusson and Kligman "maximization test". In 40% (4/10) of guinea pigs, a moderate sensitization response was observed (114).

D. Rabbits

Beside the experiments on rabbits mentioned above (19), Pecora, in 1949, (125) found a decrease of 50% in the total plasma protein in rabbits intoxicated by TNT, mainly in the albumen fraction. Experiments on the acute toxicity of TNT on rabbits and cats were carried out by Foulerton (105). Although the number of animals used was limited, a clear cut picture of toxicity was obtained, details are mentioned in the next section (4.E.)

Velling, in 1943, injected guinea pigs with TNT 0.8 g/kg subcutaneously and rabbits with 0.65 g/kg. Hemolytic anemia was observed in guinea pigs, while rabbits exhibited hypochromic anemia with acute leuko- and thrombocytopenia (117).

Jaffe et al. in 1973 (114) tested the skin irritating properties of TNT in 6 rabbits according to a modified Draize method. The TNT was prepared as a 50% paste with peanut oil. TNT was a mild skin irritant. A red pigment appeared under the skin patches 24 hours after treatment.

Using a similar procedure, 6 rabbits exhibited no observable eye irritation in eye tests with a 50% paste of TNT in peanut oil. In 24 hours following the TNT treatment, a red pigment developed around the rabbits' eyes (114).

E. Cats

To demonstrate the acute toxicity of TNT, Foulerton (105) administered this compound orally to cats and rabbits. The results were similar in both species details of those in cats are given below. In this experiment, a cat weighing 4.75 kg (sex not specified) was given 2 doses of TNT orally mixed with food. The first dose was 0.75 g given at the start and the second was 0.5 g given 24 hours later. No symptoms of toxicity were noticed after the first dose, however, 19 hours after the second dose the cat was found crouched up in its cage in a semi-comatose condition. Occasional convulsions were noted, and death occurred 49 hours after administration of the first dose. The animal lost 400 g of its weight and did not pass any urine during the last day. Postmortem examination revealed 0.5 ml of turbid, almost black, highly concentrated urine in the bladder. Sections of the liver, kidney and spleen revealed the following histopathological changes: the liver showed a slight degree of interlobular round-cell infiltration and a few globules of fat were scatttered through this liver section. Except for the Malpighian corpuscles the cortical part of the kidneys was heavily loaded with fat globules; the medullary portion, on the other hand, contained some brownish material which did not stain as hemosiderin. No pathological changes were observed in the spleen and the author attributed death to uremia due to blockage of kidney tubules.

TNT injected intraperitoneally to cats in doses of 0.10 to 0.15 g/kg caused death within 3.5 to 5.5 hours. Doses of 0.04 g/kg and over caused nervous manifestations. Histological examination revealed phagocytosis of erythrocytes, hemosiderosis of Kupffer's cells and moderate fatty degeneration of the liver (126).

F. Dogs

Vasilescu et al. (70, 71) injected 1.5 mg/kg to dogs intravenously and found a decrease in the chronaxy of the hind legs 3 to 6 hours after injection.

5. CHRONIC TOXICITY

As in the case of acute toxicity, the effect of chronic administration of TNT to various animal species is discussed below. However, microscopic changes that were noticed in various animal species are collectively mentioned in a table at the end of this section (Table IV.3).

A. Monkeys

Hart in 1974 (224) studied the oral administration of TNT in doses of 1.0, 0.1 or 0.02 mg/kg of body weight daily, seven days a week for 13 weeks (90 days). Equal numbers of male and female Rhesus monkeys, aged 44-52 months, were utilized in 3 dosage groups of 6 animals each. Appearance, appetite, motor activity, behavior, weight, blood chemistry, hematology, urinalysis, liver function testing (BSP clearance) and plasma TNT level were studied. Both the treated monkeys and 6 control animals experienced a 10% loss of body weight during the first week of the experiment, which was partially ascribed to stress. However, the controls gained back more of the lost weight than the treated monkeys. Ophthalmoscopic examinations failed to reveal eye abnormalities. Gagging and vomiting occurred on 8 occasions during the study. In the bone marrow of 2/3 of monkeys fed 1.0 mg/kg of TNT, no normal megakaryocytes were noted at necropsy. Although this finding could have been related to thrombocytopenia, no platelet counts had been made prior to sacrifice. The high dose of TNT produced greater hemosiderin deposits in liver cord cell cytoplasm as compared with hepatocyte iron-positive material in controls. The toxicological importance of the liver and bone marrow findings were uncertain. No other abnormalities were detected in TNT-fed monkeys.

B. Rats

The chronic toxicity of TNT was studied in white rats. In one experiment (115), rats were given 0.15 g/kg of TNT daily, for as long as 6 weeks, mixed with food of 3 basic diets (protein, carbohydrate, and fat diet). Only rats receiving TNT mixed with the fat diet showed signs of toxicity, which can be outlined as follows:

1. Within half an hour, a bright red pigment appeared in the urine, though animals did not appear physically ill.

2. During the first few days, a rapid loss of weight occurred, then animal weight stabilized at a new and lower level.

3. Loss of weight was associated in the first week with loss of appetite; however, appetite was regained later and became even greater than that of the control animals.

4. Within the first two weeks, animals showed signs of weakness, and anemia developed rapidly. Liver lesions, which ranged from fatty infiltration to an acute necrosis of the parenchymal cells were detected in this time. Blood changes were characterized by a decrease in hemoglobin, and the appearance of normoblasts, reticulocytes and polychromatic erythrocytes. An erythroblastic hyperplasia of the bone marrow and siderosis of the spleen were also detected.

5. About the 3rd week, animals lost hair especially between the scapulae.

6. Death usually occurred within the first month or 6 weeks.

Similar results were obtained when male albino rats were given technical grade TNT (GOST4117-67) through a gastric tube in a daily dose of 100 mg/kg for 30 days (127). Loss of weight, patchy loss of hair, hemorrhage from nasal

mucosa and miosis were observed. Authors ascribe these changes to the increased consumption of pyridine nucleotide-containing enzymes participating in the biotransformation of TNT and in the breakdown of methemoglobin. Furthermore, they recommended that vitamins B_6 and PP should be used in the treatment of occupational TNT intoxication.

To demonstrate the effect of chronic intoxication of TNT on the excitability of the neuromuscular junction, rats were given 30 mg/kg/day TNT in 2% starch mucilage orally for 60 days (128). A biphasic response in the excitability of the neuromuscular junction was observed; thus on the 15th day of intoxication excitability was reduced while on 25th to 60th it again increased. After 60 days, brain homogenates showed a reduction in the content of phospholipids and an increase in the diphenylamine reaction. These effects were weakened by the administration of vitamins B_1 and PP. Cerebral cholinesterase activity did not change, while that in blood was increased.

To study the effect of chronic TNT toxicity on the catecholamine and serotonin levels in various organs, rats were given TNT (0.2 g/kg) daily (route of administration and duration not specified). Animals showed a decrease in the norepinephrine level in heart and brain two months after the beginning of treatment (Krivitskaya and Frenkel, 1970, 129). A decrease in the serotonin level and an increase in the activity of monoamine oxidase were found in the liver and brain of rats given 100 mg/kg/day of TNT orally for 3 to 30 days (130). Moreover, these rats showed decreased levels of total protein and albumin, and increased levels of beta- and gamma-globulins in the blood serum (130).

Arzyaeva in 1966, (131) studied various methods to determine the liver function in albino rats after giving 0.8 mg/kg of TNT. The author's findings indicated that determination of bilirubin in the blood serum, and urobilin in the urine, galactose-load test, Quick test, thiopental, hexenal and thymol tests, determination of prothrombin time, detection of lipoproteins and their fractions and the bromsulfophthalein test, are helpful tools to reveal initial lesions of the liver in small laboratory animals.

C. Guinea Pigs

Bizzarri, in 1939 (124) exposed guinea pigs to TNT vapors (unknown concentration) twice a day for 4 hrs for 30 days. At the beginning of the exposure, an increase in the number of erythrocytes by 2,000,000, as well as anisocytosis, poikilocytosis and polycythemia were observed. TNT was also administered orally and percutaneously to study its effects when given by various routes.

To study the chronic effects of TNT on the nervous system, guinea pigs were given TNT by percutaneous administration (74). TNT (500 mg of 30% liniment in lanolin base) was rubbed into the skin of the back, and the animals were sacrificed on the 2nd, 6th and 11th month of poisoning. Histological sections of the brain, spinal cord, spinal ganglia, sciatic nerves, and the brachial plexus were carried out. Guinea pigs sacrificed after 2 months of treatment showed microfocal destruction of white matter in the brain and the vessels were severely enlarged and plethoric. Cocoon-shaped accumulations of lymphoid cells were detected around the boood vessels; in the perivascular spaces albuminous fluid was found. Vacuolization of the cytoplasm was found in the nerve cells of the brain stem. Those which received TNT for six months showed dystrophic processes in the nerve cells of the subcortical nuclei. The picture after

11 months of toxicity had the same character. TNT intoxication resulted in a decrease of the activity of acid phosphatase in the cytoplasm of the pyramidal cells, and an increase in alkaline phosphatase activity in the endothelium of vessels and Schwann cells.

D. Rabbits

Experiments carried out as early as 1920, by Panton and Bates (113) failed to reproduce major lesions of TNT in rabbits. However, because of the relatively high dose of TNT that was dissolved in toluene and injected subcutaneously and the negative results obtained, the details of the experimental procedures are given below:

Weight of rabbit (kg)	Dosage Schedule	Total Dose (g)	Termination of Experiment
1.49	50 mg/day for 2 months (intervals unspecified)	1.15	sacrificed
1.12	50 to 500 mg/day for 2 months	1.70	sacrificed
1.40	100 mg/day for 3 weeks	0.90	sacrificed
2.24	500 mg (one dose)	0.5	died after 10 days

No marked changes in the blood picture, no jaundice and no bile in blood serum were detected. To explain the failure to reproduce lesions observed in TNT workers in experimental animals, they assumed that poisoning might arise by a process of sensitization; the authors, therefore, injected a 1.4 kg rabbit by alternating doses of 50 and 500 mg with one dose of 750 mg at intervals of 10 days for a total dose of 2.4 g over an unspecified period. No toxic effect could be detected.

To add to the confusion, they assumed that the introduction of colon bacilli together with TNT might succeed in reproducing liver changes. Results were, nonetheless, negative.

The two major lesions of TNT intoxication that could be demonstrated by the authors were only a case of aplastic anemia induced in a cat given γ-TNT, and fatty degeneration of the liver in another cat which received TNT.

Bela (132) administered 0.5 ml of TNT (neither solvent nor concentration were mentioned) orally to rabbits during the first 80 days and then 1 ml daily for another 22 days. Animals developed anemia. Histological examination of the brain revealed microscopic hemorrhages, hemorrhagic meningoencephalitis and changes in the vessel walls. Changes were most pronounced in the thalamus and hypothalamus.

The effect of chronic intoxication with TNT on the blood picture and the blood-forming tissue was experimentally studied using rabbits that were given TNT orally for 24 to 45 days in a total cumulative dose of 0.06 to 0.12 g. Blood and bone marrow examination revealed the appearance of target erythrocytes, and a predominance of the leukopoietic activity over erythropoietic activity (124).

Pecora(125) found that rabbits chronically intoxicated by TNT showed a decrease in platelet count; no significant changes were, however, observed in prothrombin, coagulation or clot retraction times.

Zambrano, in 1951 (133) administered a weekly dose of 20 mg orally to rabbits for 4 to 6 weeks. Except for 3 rabbits, all animals died and methemoglobin appeared in the blood of all animals. The author assumed that cyanosis observed is due to capillary stasis and formation of methemoglobin (134). However, Agnisetta and Cocco (135) found that hemolytic anemia had not developed in rabbits following ingestion of TNT, yet they observed an immunological reaction associated with an increase in γ-globulin.

To determine the effect of chronic TNT intoxication on the urinary excretion of porphyrin, Fimiani, (136) administered 0.02 g of TNT per week to a group of 17 rabbits orally for 4 weeks; another group of 3 animals received 0.01 g/week for 5 weeks. An increase in the urinary coproporphyrin level which is dose-dependent was observed.

The administration of TNT orally to rabbits in a dose of 20 mg/week for 22 to 55 days resulted in loss of appetite, sluggishness and the appearance of ketonemia and ketonuria in the first day of intoxication (137).

To study the effect of chronic intoxication with TNT on plasma proteins, Pecora, in 1950 (138) administered 20 mg powdered TNT orally every 7 days to 6 rabbits. A progressive decrease was observed in the total quantity of plasma proteins (38%), in albumin (75%) and the fibrinogen fraction (25%). The globulin fraction, however, remained relatively constant. A substantial increase in urea nitrogen was also noticed. These results were interpreted on the basis of the effect of TNT on liver and bone marrow. Similar results were obtained by Coppa and Sessa (139).

Certain biochemical changes were observed in animals chronically intoxicated with TNT. Thus the effect on the glucose tolerance test was studied by Durante in 1950, (140) who found that rabbits chronically intoxicated with TNT, showed an intermediate rise in the glucose tolerance curve; during the course of toxic changes the initial values tend to increase. This effect was explained by the author to be due to the altered function of hepatic cells with the consequent failure of transformation of glucose into glycogen.

The effect of chronic TNT intoxication on the phosphatase level in blood, liver and kidneys was studied in 1949 by Aldo (118). Ten rabbits were used, 4 as controls, and 6 were administered 2 mg TNT in aqueous suspension intragastrically every 48 hrs. Phosphatase activity was determined every 10 days in blood of control and experimental animals; after 60 days animals were sacrificed; the liver and kidneys were removed and the enzyme activity in these organs was then determined. A progressive but slow decrease in phosphatase activity was noticed up to 30 days; then a more rapid decrease occurred, resulting in reduction of the enzyme activity, after 60 days, by about 50% of the control average. The phosphatase activity in the kidney was reduced by 18%; the following values were reported:

	Phosphatase activity (Kay Units)	
Organ	Control	after 60 Days
Liver	5.98	0.45 to 0.60
Kidney	1.09	0.89

TNT given subcutaneously to rabbits proved to be toxic. Though the experimental procedure was vague and the doses were not accurate, the details of

Foulerton's (105) experiments are mentioned below. Two rabbits weighing 2.26 and 2.05 kg were given TNT orally and subcutaneously, respectively. In the first case, a total of 8.4 g of TNT was given by an esophageal tube in 21 doses varying from 0.25 to 0.6 g over a period of 30 days. At the end of the experiment, the animal appeared in good health and did not lose or gain weight. A dose of 2 g TNT in vaseline was then rubbed into the shaved skin of the same animal. After 2 days two doses of 1.75 g each were rubbed into the skin on two consecutive days, after which the animal was killed and organs were examined. In the second rabbit 13 doses of TNT suspension in normal saline, varying from 0.1 to 0.45 g were injected subcutaneously during a period of 22 days. The total dose given as well as the dosage schedule were vague and confusing. The author stated that . . . "Because of the rapid separating out of the suspension in normal saline solution, the exact dosage was difficult to determine, but it was estimated that about 4.75 grams, possibly a little less, were administered by the thirteen injections". The animal, which gained 50 g by the end of the experiment, was killed and internal organs were then examined. In both cases, viz., after oral and subcutaneous administration of TNT, a dark urine, coffee-brown in color was found. The liver revealed a quantity of fat, especially in the zone arbund the central vein, and the spleen was loaded with fine and coarse granules of brown pigment. The epithelial cells of the kidney tubules of the first rabbit appeared to be swollen in the cortical zone while the pelvis of the kidney of the second rabbit that received TNT subcutaneously, was filled with a solid plug of fat.

E. Cats

Foulerton (105) in an attempt to study the chronic toxicity of TNT, administered 0.5 g of the compound mixed with lean meat or fish to a cat, 3.75 kg in weight. Although this dose was given every third or fourth day for three weeks, the exact amount of ingested TNT could not be estimated because an attack of sneezing and salivation followed swallowing a mouthful of food. Because the animal appeared in perfect health and did not lose any weight, the author administered 1 g of TNT mixed with vaseline by rubbing thoroughly into shaven skin, on each of 3 consecutive days and then 3 more doses were rubbed into the skin with two days' interval, so that 6 doses (1 g each) were applied during a period of 12 days. On the 13th day the animal was very drowsy and appeared to be moribund. Its condition improved the following day, so another dose of 1 g was applied each on the 15th and 16th days, and on the 17th day a dose of 1.5 g was rubbed. Thus, a total of 9.5 g had been administered. On the 18th day the animal died, after losing 2.6 kg of body weight. The urinary bladder was full of urine which on centrifugation, gave a dense sediment of mucus containing a very large number of fat globules. Kidneys and liver showed fatty infiltration, while heart, lungs and spleen showed brown pigments which did not stain as hemosiderin.

F. Dogs

To study the effects of TNT toxicity on various organs, Kramer and Meierhof (141) administered TNT (amount not specified) to dogs, either orally (in butter), by skin inunction (in lard) or by subcutaneous injection (in olive oil). The following symptoms were observed (regardness of the route of administration used): diarrhea, weakness, emaciation and leukocytosis. Vomiting was observed only in dogs given TNT orally, apparently due to direct irritation of the stomach. The most outstanding autopsy finding is a moderate central degeneration of liver cells, with congestion of the capillaries. Three years later, Voegtlin et al. (112) administered 5 to 33 mg/kg TNT orally to dogs for long periods (not specified). The symptoms which developed included cyanosis, sali-

vation, diarrhea; incoordination, icterus and anemia. Cyanosis usually developed at the beginning of intoxication while weight loss was unnoticed until shortly before death. In another study, eight female dogs weighing between 5 and 11 kg were maintained on a normal diet of ground horse meat and dog biscuits and were divided into 4 groups each of 2 animals. TNT was given orally, daily except on Sundays for 4 weeks, in a dosage level of 5, 15, 25 and 100 mg/kg. Practically all animals showed the same symptoms, which appeared after the second dose in groups that received 15, 25 and 100 mg/kg and after the third dose in the group that received 5 mg/kg. During the first 7 to 10 days, dogs appeared extremely sick, were ataxic, manifested asynergia and incoordination, developed irregular and involuntary eye movements, had diarrhea and passed very dark urine. By the end of the second week all animals showed marked improvement with a return towards the normal state (142). In further study by the same group of authors, 50 mg/kg/day TNT was administered orally to dogs for 12 weeks. They found that this dose, which produced maximum symptoms of chronic poisoning in dogs, did not prove fatal during the 3 month duration of experiment, did not cause depletion of ascorbic acid from blood plasma nor did it increase the excretion of ascorbic acid in the urine (143).

In a more recent study by Hart in 1974 (223), three groups each of 6 adult beagles (9 males, 9 females) were given daily dosages of TNT of 0.02, 0.1 or 1.0 mg/kg of body weight per day for 90 days. Temporary episodes of vomiting subsided after the first 2 weeks of the experiment. Appearance, behavior, appetite, weight, blood chemistry, hematology, urinalysis and gross and microscopic examination of organs following necropsy, revealed no signs of toxicity. There were no observable differences between treated dogs and 6 male and female control beagles.

In an attempt to induce TNT intoxication by inhalation, Van Oettingen et al. (1944, 120) exposed four dogs, 5 days/week for 2 weeks to vapors produced from 50 mg/kg body weight of TNT; then for an additional two weeks to vapors produced from 100 mg/kg body weight. Generally animals were in good shape, and except for 2 dogs which lost 0.8 and 1 kg, no weight loss was observed. The blood picture was essentially the same before and after the exposure period. The authors found it very difficult to volatilize TNT in sufficient quantities to produce systemic toxic effects in dogs. For this reason, in a later communication (144), they administered TNT by insufflation to 2 series of dogs each of 3 animals, that received doses of 25 mg/kg and 50 mg/kg, respectively, 5 days/week for 17 weeks. Both groups showed essentially the same symptoms which consisted of a steady loss of weight of up to 6 kg, salivation, vomiting, diarrhea, weakness and incoordination. Moderate anemia, characterized by a reduction of the red blood cells and of the hemoglobin, from which they recovered after some weeks despite continued exposure, was noticed. No bile pigments, urobilinogen, or sugar were detected in urine; only some albumin and occasionally blood were detected in the urine, but only towards the end of the exposure.

Kleiner (145) in an attempt to determine the external secretory function of the liver in chronic TNT intoxication, injected TNT to white dogs subcutaneously with 20 to 50 mg/kg/day every other day for 3 months, and found suppression of bile formation and bile secretion.

In regards to the effect of TNT on gastric secretion, chronic administration (2 1/2 years) caused an increase of phosphates, ammonia and lactic acid content of the gastric juice of dogs given 0.1 to 1 and 5 to 20 mg/kg. Morphological changes similar to exfoliative gastritis were also observed (146, 147).

G. Microscopic Changes in Experimental Lesions

No remarkable changes were observed in the brain, heart and skeletal muscles of guinea pigs and rabbits intoxicated with TNT (19). Lungs, however, were edematous and showed hemorrhage in some of the lobules. Large phagocytic endothelial cells were found in the alveoli of guinea pigs in which TNT was forced into the lungs. The liver showed fatty changes and diffuse areas of necrosis. Microscopic changes in the spleen were in the form of enlargement of blood sinuses which contained large phagocytic endothelial leukocytes which were filled with golden pigments, blood fragments and whole red cells. Changes in adrenal glands were not remarkable, though in some instances groups of medullary cells had entirely disappeared and vacuoles filled with free red cells were found instead. Kidney changes were manifested by congestion of the glomeruli and dilatation of the glomerular spaces which were filled with cellular debris, free pigment granules and occasionally with red blood cell ghosts.
Bone marrow contained megakaryocytes and multinucleated giant cells which were numerous and often appeared arranged in groups of three or more.

Microscopic changes that were noticed in various animal species are listed in the following table IV.3 (148).

TABLE IV.3. MICROSCOPIC CHANGES DUE TO CHRONIC TNT TOXICITY

Species	Dose and Route of Administration	No. of Doses	Liver	Spleen	Kidney
Rats	0.3% orally		Slight fatty changes mainly midzonal, periportal or centrolobular. Kupffer cells contained hemosiderin.	Very marked hemosiderosis in pulp reticuloendothelial cells	Rare traces of iron-positive pigments
Guinea Pigs	200 mg/kg orally then 400 mg/kg	16 43	Slight to marked centrolobular fat droplet deposits in liver cells and moderate to marked Kupffer cell hemosiderosis.	Moderate to marked pulp hemosiderosis and pulp myelosis.	Hemoglobin casts in the collecting tubules. Moderate fatty infiltration of the epithelium of the convuluted tubules.
Rabbits	200 mg/kg Subcutaneously	9-29	Slight to moderate centrolobular fatty degeneration of the liver.	Quite marked pulp hemosiderosis but little or no myelopoiesis	Numerous hemoglobin casts in the collecting tubules, and marked hemosiderosis of the cortical tubule epithelium.
Cats	50 mg/kg Subcutaneously	4-6	Slight to moderate centrolobular fine fat droplets, some centrolobular congestion and atrophy of cell cords, slight to marked Kupffer cell hemosiderosis.	Slight to marked myelosis, and pulp hemosiderosis	Fatty infiltration of the cortical convoluted tubules.
	20 mg/kg Subcutaneously	10-30 21-30	Fairly marked hemosiderosis of Kupffer cells	Hemosiderosis of the pulp reticuloendothelium, - Slight splenic myelosis	Occasional hemosiderin in renal convoluted tubules

Ref.: Lillie (148).

V. CARCINOGENICITY, MUTAGENICITY AND TERATOGENICITY OF TNT

To date, no carcinogenic effect has been reported from TNT intoxication among workers, and no tumors have been found in postmortem examinations of any persons chronically intoxicated with TNT.

However, an animal investigation concerning carcinogenicity was reported by Schepers, in 1971 (149). He endeavored to induce specific pulmonary lesions, including lung cancer, by atmospheric exposure to several compounds including TNT. No neoplastic effect was demonstrated in guinea pigs, rats or mice exposed to TNT aerosols for an average of 24, 18 and 15 exposures/month, respectively (further details were not provided).

After 6 months of topical application of 30% TNT 5 times a week to 20 Wistar rats, tibial bone marrow cells from 50% of the animals exhibited chromosomal changes. In 20% of observed mitoses in affected rats, chromatid changes, chromosome breaks and dislocations were observed, although changes in chromosome number were not found. The authors suggested the possibility of anomalies in future generations of these rats (222).

The mutagenicity of TNT (explosive grade) was studied by the Ames assay system using histidine-requiring strains of *Salmonella typhimurium*. In concentrations of 0.5 to 10 µg/ml of overlay, TNT is a frameshift mutagen characterized by a linear mutagenic response curve. Concentrations above 10 µg/ml inhibited the growth of the test bacterium. Moreover, TNT accelerates the reversion rate of a frameshift tester, TA-98. On the other hand, the major microbial metabolites of TNT appeared to be nonmutagenic (150).

No studies have been hitherto reported on the teratogenicity of TNT.

VI. RELATIONSHIP BETWEEN DIETARY FACTORS AND TNT TOXICITY

Disability arising among munition workers from exposure to TNT presented an industrial health problem of major importance during World Wars I and II. Loss of working time and the incidence of fatal cases has urged the study of measures which may throw light on the factors which influence susceptibility to TNT toxicity. Without evidence of the protective role of nutrition against TNT toxicity, the manager of an ordnance plant in England, in 1917, after noting that female employees complained from gastrointestinal upsets, undertook a nutrition program that resulted in a decreased incidence of gastrointestinal disorders from 11.6% to less than 2.0% (151). Since that time, high carbohydrate diets, ascorbic acid, calcium and milk have been recommended for prophylaxis against TNT toxicity. An analysis of the effect of diet on TNT toxicity will, therefore, be presented in the following section. Topics discussed include experimental investigations in animals and observations in humans.

1. EXPERIMENTAL INVESTIGATIONS IN ANIMALS

Himsworth and Glynn (115) believed, from experiments with rats, that the nature of diet affects the susceptibility to TNT toxicity. Furthermore, several factors such as vitamin C, methionine, vitamin B complex and several others were advocated for the prevention and treatment of TNT toxicity. For this reason, the effect of the nature of food per se, as well as several additives will be discussed.

A. Effect of the Nature of Food

Experiments were performed to show whether or not the type of food given to animals has a favorable effect in preventing TNT toxicity.

i. Meat: In 1919, Voegtlin et al. (112) found that dogs fed a meat diet are more resistant to TNT toxicity than dogs which are fed white bread and milk. They suggested, therefore, that TNT workers should eat, besides other nourishing food, at least 150 to 200 grams of meat daily.

ii. Milk: Consumption of milk has been recommended for the prevention of TNT intoxication and it is believed that milk may be helpful in alleviating the gastric irritation experienced by some TNT workers. To answer the question whether or not the addition of milk to a well-balanced basic diet has a favorable effect on dogs against TNT intoxication, Donahue et al. (152) studied the effect of the addition of 300 ml of milk to the diet of dogs that were given 50 mg/kg TNT orally. They found that the addition of milk alleviates only slightly the toxic effects produced by TNT. Neither did they find any evidence that the addition of milk promotes the detoxification of TNT. However, animals on a milk diet lost less weight than the control group; the inflammatory reactions in the intestinal tract was less severe, and the intestinal mucosa of the milk-fed dogs was covered with a fibro-mucoid film that is absent in control animals. Though giving extra milk to munition workers is questionable in view of the fact that a high fat diet increases TNT toxicity, the authors concluded that milk, by its demulcent action, protects the mucous membranes of the gastrointestinal tract and may interfere with the absorption of TNT from the intestine.

iii. Fat-, carbohydrate- and protein-rich diet: In 1942, Himsworth and Glynn (115) demonstrated that the nature of diet greatly influences the susceptibility to TNT intoxication. They used white "Wistar" rats to which TNT was given orally (by stomach tube or mixed with diet) or parenterally. In all cases TNT was administered as oily solutions: given intragastrically or parenterally 5% solution in arachis oil was used; given with diet, the amount of TNT required was dissolved in part of the fat of the diet; if the diet did not contain fat, the TNT was dissolved in the minimum necessary quantity of arachis oil and then mixed with the food. The dose of TNT given daily was 0.15 g/kg body weight and experiments lasted for 70 to 100 days. Three basic diets were used: one high in fat, another high in carbohydrate and a third high in protein. They found that the nature of the diet has a great influence on the development of toxic manifestations in rats. Thus animals on a high fat diet showed severe symptoms and marked pathological lesions compared to animals taking a high carbohydrate or a high protein diet where ill effects were slight or even absent. The authors ascribe the increased toxicity of TNT in the presence of fat diet, to the "fat diet so influencing the animals' metabolism as to impede its ability to dispose of the TNT within its tissues". Shils and Goldwater (151) do not share this opinion; they think that the low protein content of the diet might be as important or even more important than the high fat intake. This viewpoint is supported by the results of Smith et al. (153) who found that cystine supplements given to TNT treated rats afforded some protection, and even greater improvement was noted with methionine or increased amounts of casein.

A few years later, Goldwater and Shils (154) studied the effects of fat and protein contents of diet on the toxicity of TNT. They found that TNT (150-300 mg/100g diet) depresses growth of male rats regardless of the type of diet used. However, increased protein intake is reflected by an equal increment in weight increase both in control rats and TNT-treated ones. They concluded, therefore, that TNT does not seem to exert its deleterious effect by interfering with the protein metabolism involved in growth. On the other hand, the same authors reported that a high fat intake was somewhat deleterious to rats fed TNT.

B. Effect of Other Food Additives

Several experiments were performed to study the protective effect of several substances added to the diet of experimental animals, against the toxic effects of TNT. Among the substances studied were vitamin C, methionine, vitamin B complex and nicotinamide.

1. Vitamin C (Ascorbic Acid)

The susceptibility of different species of animals (including man) to the toxic actions of TNT varies considerably. Thus while dogs and cats(and probably man) are highly susceptible to TNT toxicity, white rats, guinea pigs and rabbits exhibit high natural resistance to it (112, 153). Assuming that ascorbic acid might antagonize the toxic effects of organic nitro compounds (through the activation of catalase, which by yielding oxygen would tend to inhibit their reduction), Smith et al.(153) carried out experiments to ascertain the effects of ascorbic acid in chronic TNT toxicity. They selected various animal species: cats (highly susceptible to TNT toxicity), and animals with natural resistance, e.g., rats (require no exogenous ascorbic acid), guinea pigs (exogenous ascorbic acid is indispensable), and rabbits (intermediate). Their findings are summarized in table VI.1.

TABLE VI.1. EFFECT OF ASCORBIC ACID ON TNT TOXICITY

Species	Dose of TNT and route of administration	Diet and ascorbic acid content (or dose)	Duration of administration (days)	Average weight changes	Average Hb (g/100 cc)	Percent fatty livers	Conclusion
Rats (white Wistar)	0.3% in semi-synthetic diet, orally (ad libitum)	5% casein	60	-33 g	12.4	70	Ascorbic acid failed to have favorable effect.
		5% casein + 2% ascorbic acid	70	-44 g	11.2	50	
		18% casein	200	+59 g	14.0	20	
Rabbits	0.2 g/kg subcutaneously, every other day as a 10% solution in olive oil.	"Purina" rabbit chow	60	—	10.6	25	No demonstrable beneficial effect from ascorbic acid.
		Control (no ascorbic acid)					
		treated 0.2 g/kg ascorbic acid subcutaneously every day	60	—	9.9	29	
Guinea pigs	0.2 g/kg by stomach tube daily except Sundays; after 16 doses, TNT dose increased to 0.4 g/kg. as 10% solution in olive oil with 20% acetone	"Purina" rabbit chow					No beneficial effect from ascorbic acid treatment.
		control (0.01 g ascorbic acid subcutaneously twice a week)	42	—	13.6	85	
		treated (0.1 g ascorbic acid subcutaneously daily)	42	—	12.9	92	
Cats	0.02 g/kg daily subcutaneously as 10% solution in acetone-olive oil	Control-raw lean meat	17	-800 g	2.5	43	Some beneficial effect of ascorbic acid was observed.
		treated (ascorbic acid was given subcutaneously in doses of 0.2 g/kg daily)	17	-800 g	1.2 (aver. loss)	40	

It was concluded from this table that ascorbic acid had little effect on the course of chronic TNT toxicity in rats, guinea pigs or rabbits. In cats, which are relatively highly susceptible to TNT toxicity, ascorbic acid appeared to have, however, a slightly favorable influence on the course of intoxication. Neither did ascorbic acid affect the distribution and elimination of reduced degradation products of TNT. Similar results were obtained in dogs fed 150 mg of ascorbic acid and 50 mg/kg powdered TNT daily (152). No significant protective effect of Vitamin C was observed in guinea pigs (155).

ii. Methionine, B-Complex, Liver Extract and Nicotinamide

In an attempt to prevent the pathological changes induced by TNT, various compounds were administered to rabbits poisoned with 0.02 g of TNT once a week for 4 weeks. Five groups of rabbits were used. The first group was untreated and received TNT only. The other 4 groups received the following compounds, respectively: methionine (0.25 g), vitamin B complex (0.5 mg synthetic vitamin B_1, 0.3 mg vitamin B_2, 0.15 mg vitamin B_6, 7.5 mg nicotinamide and 15 mg calcium pantothenate), fresh liver extract (from 1 kg of liver), and 15 mg of sodium nicotinate. The degenerative changes in the liver induced by TNT were inhibited and to a lesser extent in the kidney, adrenals, spleen, lung and heart (156).

These results were confirmed by Fairhall, a year later (157) who found that rabbits chronically intoxicated with TNT showed degenerative changes in the liver, kidney and spleen. These effects were not so apparent in animals pretreated with vitamin B complex, liver extract or methionine.

2. OBSERVATIONS IN HUMANS

A. Vitamin C

Waterman et al. (158) reported a case where the red dye in the urine of a TNT worker had disappeared within 48 hrs after the administration of vitamin C. They believe that munition workers can be protected from toxic effects of TNT by prophylactic administration of adequate amounts of vitamins.

B. Alcohol

Putnam and Herman (90) reported about three workers that took one or two small drinks in TNT plants; this resulted in dizziness, and often cyanosis and fainting. Their pulse and respiration became rapid. The authors attributed the increased toxicity of TNT by alcohol to the excellent dissolving power of the latter; and they concluded that "TNT and whiskey don't mix". This finding is not in agreement with that of Teisinger (159) who found that alcohol, consumed in the form of beer, increased the oxidation of TNT in the tissues of 34 persons who had worked with TNT for a period of one to 15 years.

VII. ABSORPTION, DISTRIBUTION, BIOTRANSFORMATION EXCRETION, AND BIODEGRADATION OF TNT

To produce its characteristic effects (pharmacologic or toxicologic) a substance must be present in appropriate concentrations at its site(s) of action for a suitable length of time. This concentration depends not only on the given amount (dose) of the compound, but also upon the extent and rate of its absorption from the site of administration, distribution, binding to certain tissues, biotransformation and excretion. This section, therefore, reviews the absorption, distribution, biotransformation and excretion of TNT.

1. ABSORPTION OF TNT

TNT gains access to the body through lungs, gastrointestinal tract, and/or the skin, which is the chief avenue of absorption (90). Putnam and Herman (90) reported that "poisoning via the respiratory tract rarely, if ever, occurs". As demonstrated by Voegtlin et al. (110) skin absorption takes place most readily through the palms of the hands, the neck and the face, in the order given. Oily skin and excessive sweat were reported to favor absorption (12).

TNT is ingested by mouth through the negligence of certain workers who eat lunch on the premises with contaminated hands. Also, because workers cannot smoke they tend to chew tobacco handled with dirty fingers, which constitutes another source of TNT access to the stomach (90). The most important path of absorption, however, is the skin and therefore preventive measures should be aimed largely at avoiding contact with TNT.

It is the belief of Voegtlin et al. (110) that TNT may be retained in the organism for a considerable period of time, as indicated by the progressive anemia after single administration, and by the slow recovery of animals.

Several experiments were carried out to determine the rate of absorption via several routes of administration, and results revealed that TNT is readily absorbed when given orally, when administered subcutaneously dissolved in oil or when introduced as dust into the lower air passages. Details are as follows.

A. Absorption Through the Skin

In an attempt to demonstrate that TNT can be absorbed via skin, Haythorn (19) rubbed large quantities of TNT powder on his arms for several days consecutively. Except for a reddening of the skin, no other ill effects, not even a positive Webster's test, could be demonstrated. However, guinea pigs and rabbits that were rubbed with 10% TNT in lanolin showed a positive Webster's test and liver lesions after several innunctions.

Several years later, in order to study the absorption of TNT through the intact skin of swine, Neal et al. (160) suspended 2 g of TNT in 3 ml of glycerol and applied it to the clipped skin of shoats, covered it with gauze and strapped it to the body with adhesive tape. The urine was collected daily, and was positive for 2,6-dinitro-4-aminotoluene for 8 days. Amounts determined ranged from 0.002 to 0.014 mg/80 ml of urine. They concluded that TNT can be absorbed even through the dry scaly skin of pigs. The same group of authors (161), studied absorption of TNT through the skin of humans by rubbing 500 mg of

finely powdered pure TNT into the palms of both hands of 2 human subjects, which were covered with rubber gloves for 8 hours. Traces of the metabolite 2,6-dinitro-4-aminotoluene were found in the urine samples collected during the period of exposure, and for 15 hours thereafter.

B. Absorption via the Respiratory System

Haythorn, in 1920-21, (19) conducted several sets of experiments to determine the routes of absorption of TNT. In the first series he exposed 4 guinea pigs to fumes of volatilized TNT for 3 hours a day for 30 days. Animals died from the heat used to volatilize TNT, but no lesions which could be ascribed to TNT were observed. The lack of toxicity was explained on the grounds of breakdown of TNT to several different gases on vaporization. Another series of experiments was then carried out in which a fine powder of TNT was introduced into the lungs of experimental animals. No details were given about the experimental procedures and the results were negative. The author concluded that lungs, as a route of intoxication in TNT workers, are "an unimportant one".

Another set of experiments was carried out by Von Oettingen et al. (144) who administered powdered TNT by insufflation to dogs in a dosage schedule of 25 to 50 mg/kg for 5 days/week. They found that 75% of the dose was absorbed from the respiratory tract. After 17 weeks of exposure, no considerable amount of TNT, nor its metabolite 2,6-dinitro-4-aminotoluene, could be detected in the trachea, lungs, spleen, gut, liver, bile, kidneys or the bladder of the dogs. They concluded that TNT is not retained to any considerable extent in these organs.

C. Absorption along the Alimentary Canal

That TNT dust can reach the alimentary canal is demonstrated by the fact that TNT workers usually complain of a bitter taste in the mouth. Moreover, TNT is very soluble in saliva and in gastric juice; the dust which collects in the posterior nasopharynx is commonly swallowed. Experimentally, guinea pigs fed TNT and milk developed diarrhea, and poisoning symptoms were apparent in from 3 to 14 days (121). Horecker and Snyder (162) carried out experiments on two human subjects who received daily doses of 1 mg/kg TNT for 4 successive days. About 3% of the total amount of TNT could be recovered from the urine in the form of 2,6-dinitro-4-aminotoluene.

D. Solubility of TNT in Body Fluids

TNT is soluble in various body fluids to various degrees. Consequently the degree of TNT toxicity to various tissues depends upon the extent to which TNT is soluble in that particular tissue. Haythorn (19) found that TNT is soluble in the following body fluids as shown by a positive Webster's test: saliva, stomach juice, (cow's) milk, human bile, human serum and urine.

In summary, there are 3 possible routes of entrance of TNT to the body of workers exposed to that compound. Though TNT is mainly absorbed through the skin, it can gain access via the air passages, or by the alimentary tract, depending on the type of the exposure involved. Thus, volatilized TNT, or TNT dust is inhaled by workers in the nitrating room or grinding department, respectively. Contamination of skin usually occurs where workers handle and package wet TNT.

2. DISTRIBUTION OF TNT

The distribution and excretion of radiolabeled TNT was studied after oral administration to rats (114). Animals were killed 30 minutes or 24 hours after the administration of TNT and results are summarized in Table VII.1.

3. BIOTRANSFORMATION OF TNT

Based upon several *in vivo* and *in vitro* experiments, Alperin et al. (29) concluded that the liver is the major site of detoxication of TNT. Several investigators studied the fate of TNT either after administering it to intact animals or by adding it to slices or homogenates of various tissues. Results of both the *in vivo* and the *in vitro* experiments are in agreement, and details are as follows:

A. *In Vivo* Experiments

The biotransformation of TNT has been extensively studied since the beginning of this century. Thus, Moore (1917), Voegtlin et al. (1920), and Dale (1921) have found tetranitroazoxytoluene and an aminodinitrotoluene in the urine of both workers exposed to TNT, and animals receiving TNT orally or by injection (163). Further investigations showed that TNT is detoxified either by reduction or by oxidation processes.

1. Reduction

The existence of the reduction mechanism, involving a single nitro group, was proven by Channon et al. (164) in 1944 who isolated 2,6-dinitro-4-hydroxylaminotoluene(I), 2,6-dinitro-4-aminotoluene(II), and 2,4-dinitro-6-aminotoluene(IV), from the urine of rabbits that received TNT orally. They postulated that the first step in the reduction of the nitro group is the production of the hydroxylamine derivative, as described in Figure VII.1.

Although they failed to isolate the compound 2,4-dinitro-6-hydroxylaminotoluene (III), the isolation of its further reduction product (IV), led them to assume that the formation of compound III is reasonably certain. Only 30% of the administered TNT was found as 2,6-dinitro-4-aminotoluene (II). It is noteworthy that the compound 2:2':6:6'-tetanitro-4:4'-azoxytoluene (V) that has been isolated or detected in the urine of humans and animals exposed to TNT, and that was formerly believed to be excreted, is not a TNT metabolite. Rather, it is readily formed from 4-hydroxylamino-2,6-dinitrotoluene (I) in solution; Channon et al. (164) have shown that it is absent from freshly voided urine of rabbits given TNT, but is formed from dinitrohydroxyaminotoluene during the extraction procedure. One year later, in 1945, Lemberg and Callaghan (165) carried out experiments on rats and with the urine of munition workers. They found that the main metabolites in human urine are the same as these found in rabbit's urine by Channon et al., namely I, II and IV. However, in rat's urine they found in addition to I, II and IV, 2,4-diamino-6-nitrotoluene (VI) and 5-nitrophenylene diamine (VII), providing the first evidence of demethylation of TNT in vivo. They also found that 20% of a single oral dose of TNT is excreted in the urine of rats as diazotizable aromatic amino compounds. Human volunteers, however, excreted on the average 40% of small doses (10 to 30 mg) given orally as aromatic amino compounds in the urine (166,167).

TABLE VII.1.

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE ORAL DOSE OF TNT (RING-UL-^{14}C)

	% of Administered Dose		Tissue /Plasma Radioactivity Ratio[e]	
	30 Minutes[c]	24 Hours	30 Minutes	24 Hours
Gastrointestinal tract plus contents	-	20.7 ± 2.7	-	-
Feces	-	5.5 ± 1.1	-	-
Whole Blood[a]	0.2 ± 0.0[d]	0.6 ± 0.1	-	-
Expired Air	-	0.1 ± 0.0	-	-
Urine	0.2 ± 0.1	53.3 ± 3.1	-	-
Liver	0.3 ± 0.1	0.6 ± 0.0	3.5 ± 0.6	2.0 ± 0.1
Kidneys	0.1 ± 0.0	0.2 ± 0.0	2.4 ± 0.2	2.7 ± 0.2
Brain	0.1 ± 0.0	< 0.1	1.3 ± 0.2	0.3 ± 0.0
Lungs	-	< 0.1	-	0.9 ± 0.1
Skeletal Muscle[b]	-	1.0 ± 0.1	-	0.4 ± 0.0
Recovery		82.1 ± 3.0		

a. Based on 7.0% of the body weight.
b. Based on 40% of the body weight.
c. The rats in the 30-minute group received the minimum lethal dose.
d. Mean ± S.E. of three rats.
e. Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

Ref.: Lee et al. (114)

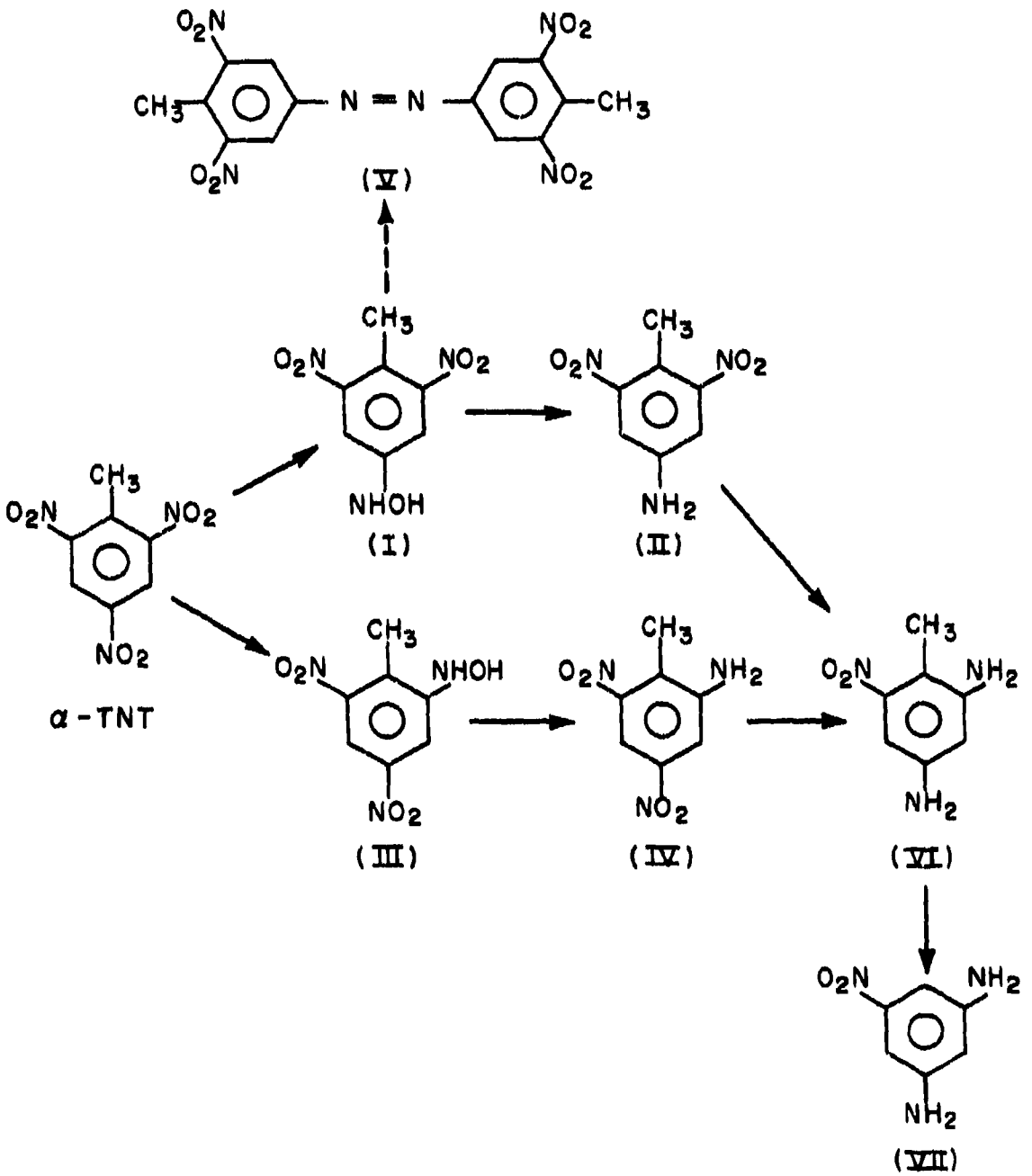


Fig. VII.1. REDUCTIVE BIOTRANSFORMATION OF TNT.

ii. Oxidation

In addition to undergoing reduction, TNT may also be metabolized by oxidation to give trinitrobenzyl alcohol (VIII), and trinitrobenzoic acid (IX) as illustrated in Figure VII.2.

This oxidation mechanism is only hypothetical and is based upon the following indirect evidence of Channon et al. (164):

In an effort to determine the nature of the red pigment present in TNT urine, 15 possible intermediate substances including α-, β- and γ-TNT were administered to rats. Only α-TNT and 2,4,6-trinitrobenzyl alcohol (VIII) caused red pigment excretion. The alcohol may thus be a constituent or a precursor of the red pigment.

Rabbits fed with trinitrobenzyl alcohol, excreted a diazotizable substance in urine, which was probably an aminodinitrobenzyl alcohol. About 47% of the TNT administered was excreted in urine as glucuronides. Since glucuronic acid is found naturally combined only with substances possessing an aliphatic or aromatic hydroxyl group, glucuronides of TNT in urine must arise from a TNT oxidation product, viz., trinitrobenzyl alcohol (164).

Further support was obtained by the finding of Lemberg and Callaghan (165) that rats fed with TNT excreted 5-nitrophenylenediamine (VII) in the urine. The formation of this compound involves the loss of a methyl group which, according to Williams (163), could conceivably occur by oxidation of TNT to 2,4,6-trinitrobenzoic acid (IX) via the alcohol. Decarboxylation and reduction will then result in the formation of (VII).

More evidence for the occurrence of oxidation processes, though not involving the methyl group, has been provided by Lemberg and Callaghan (165), who isolated an aminonitrocresol (X) from the urine of rats fed with TNT. However, they have not elucidated the mechanism of its formation.

On the other hand, Snyder (168) was not able to demonstrate the presence of 2,4-diamino-6-nitrotoluene (VI), 2,4,6-triaminotoluene, 2,4,6-trinitrotoluene, 2,4,6-trinitrobenzyl alcohol (VIII), 2,4,6-trinitrobenzaldehyde or 2,4,6-trinitrobenzoic acid (IX) in the urine of dogs which received TNT orally.

In summary, TNT has been found to be metabolized by man and rabbits to 2,6-dinitro-4-hydroxylaminotoluene, 2,6-dinitro-4-aminotoluene, and 2,4-dinitro-6-aminotoluene. Rats, however, convert TNT, in addition to the above-mentioned metabolites, to 2,4-diamino-6-nitrotoluene and 5-nitrophenylenediamine.

B. *In Vitro* Experiments:

In 1946, Bueding and Jolliffe (169) studied the metabolism of TNT in vitro, using liver and muscle slices and heart extracts. They found that TNT is metabolized to an intermediate compound which inhibits tissue respiration; this compound is further metabolized to a substance that does not inhibit the oxygen uptake of tissues. Using liver extracts, 4-amino-2,6-dinitrotoluene (II) was identified as the end product of the metabolism of TNT. Under anaerobic conditions, the rate at which TNT was removed by tissue slices and homogenates is more rapid than under aerobic conditions. TNT was found to be metabolized by

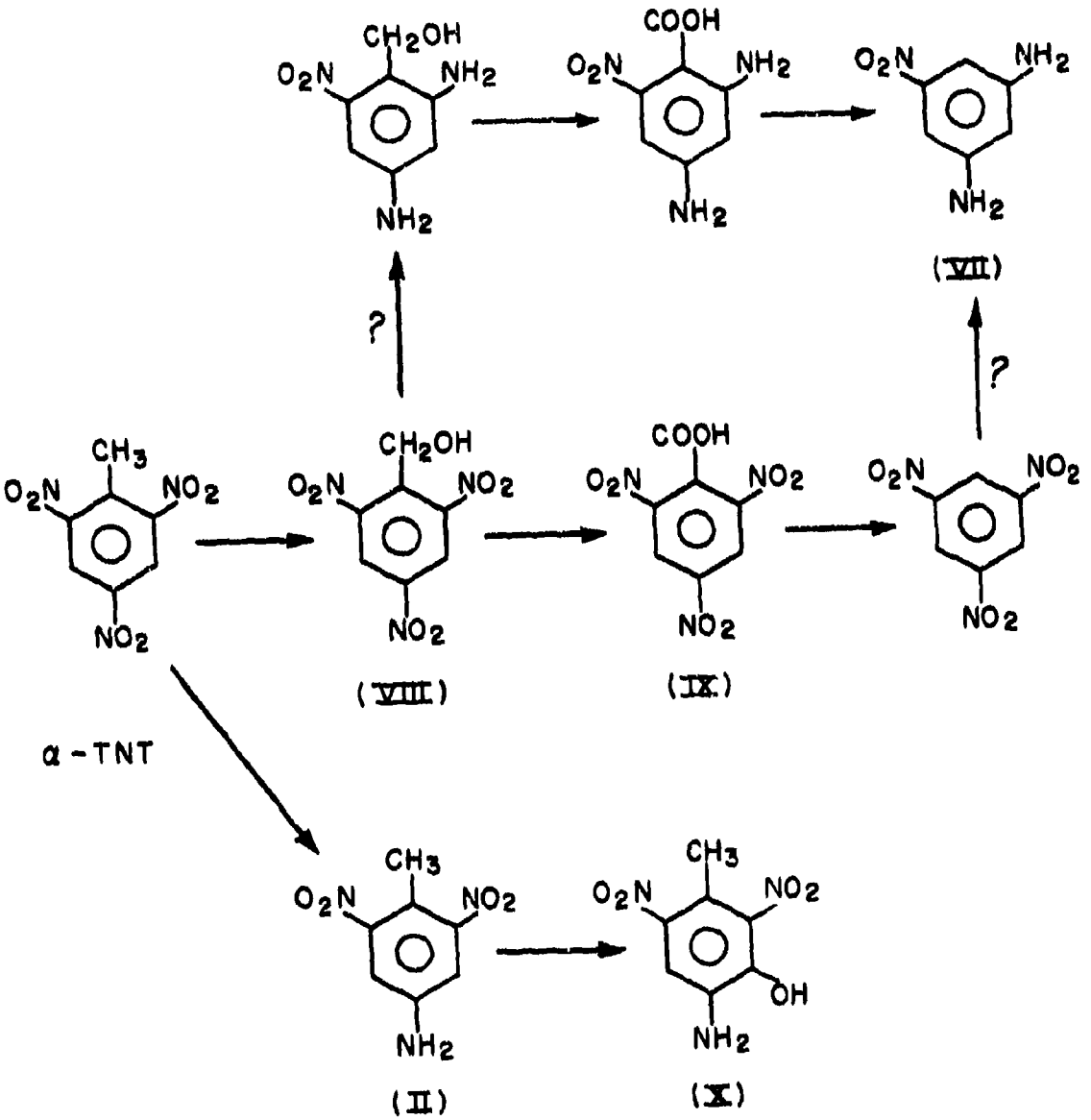


Fig. VII.2. OXIDATIVE BIOTRANSFORMATION OF TNT.

a system containing reduced diphosphopyridine nucleotide (DPN) and a highly purified flavoprotein (Straub). This is brought about by a transfer of hydrogen from flavoproteins to the nitro group. It is also reduced by partially purified xanthine oxidase to hydroxylaminodinitrotoluene.

4. EXCRETION OF TNT

Based upon Webster's test, urine is undoubtedly the main route of excretion of TNT. Voegtlin et al. (110) claimed that TNT is also excreted in bile; however, this is in contradiction with the findings of Haythorn (1920-21, 19) who could not obtain a positive Webster's test in the feces of any animals that had been given TNT by any other route except orally.

Horecker and Snyder (162) administered 1 mg/kg TNT/day orally for 4 successive days to humans and found that the daily excretion of 2,6-dinitro-4-aminotoluene (II) and 2,4-diamino-6-nitrotoluene (VI) in the urine amounted to 3% of the ingested TNT, and fell almost to zero within 24 hours following the last dose.

5. MICROBIAL DEGRADATION OF TNT

In 1965, Fowler (170) stated that "during periods of heavy production, as much as 10 million pounds of TNT have been produced per day in the U.S.A. alone", and "for every pound of pure TNT manufactured, approximately 0.5 gallon of solution containing about 0.04 pounds of TNT isomers is formed as waste". It is also a common practice at bomb and shell loading sites to wash down equipment by hot water and steam, and to dispose of TNT waste by draining it into the nearest stream. The solubility of TNT in water is 130 mg/l at 20°C, (see section VII) and hence a substantial amount can be carried by water streams. Since about 2 mg/l of TNT in water can adversely affect the aquatic life, and since it was recommended that the TNT level in water for human consumption should be kept below 1 ppm (170), various methods for the disposal of TNT wastes have been the subject of several studies (171, 172, 173). Among these methods is the evaporation of TNT wastes in open basins which results in pollution of ground water and the accumulation of TNT "syrups", and the dilution of TNT wastes with massive amounts of water, which is often not available. Disposal of TNT wastes by passing them directly through municipal sewage treatment plants, i.e., by degrading it biologically, or by the use of oxidation ponds are other alternatives. Mention, therefore, of biodegradability of TNT, is mandatory, in order to shed some light on one of the most economical ways to minimize water pollution due to military munition products. Furthermore, the findings of Lemberg and Callaghan (165) that rats given TNT orally excreted 5-nitrophenylenediamine (VII), show that in this particular species, certain microorganisms might be present among the intestinal flora which are capable of demethylation, or oxidative decarboxylation of TNT to give VII. This might be also the reason for the low susceptibility of rats to TNT poisoning.

The presence of TNT wastes in water streams is evidenced by a brick red color imparted to the stream due to the formation of a TNT complex of unknown composition. It has been suggested that the formation of this colored TNT complex involves photochemical (see section VII) and biochemical reactions. In a study of the role played by the putative decomposition reaction, Grilng (172) demonstrated that *Escherichia coli* is capable of reducing at least one of the nitro groups of TNT to its respective amine. Furthermore, the author found that this reduction process is enzymatic and requires the reduced pyridine nucleotide, NADH for enzymatic activity. These findings were confirmed by Saz and Slic (174).

Three yellow pigment-producing pseudomonas-like organisms isolated from mud and water samples have been shown to metabolically oxidize TNT (170, 171). Metabolites that were found in the media are in the following descending order: 2,2',6,6'-tetranitro-4-azoxytoluene, its isomer 2,2',4,4'-tetranitro-6-azoxytoluene, 4,6-dinitro-2-aminotoluene, 2,6-dinitro-4-hydroxylaminotoluene and nitrodiaminotoluene. The authors suggest that this finding gives possibilities to the solution of TNT waste disposal problems.

Fowler (170) studied biodegradation of TNT by two different organisms isolated from rat feces and raw sewage which are termed *Pseudomonas* I and II. *Pseudomonas* I brought about the most rapid degradation of TNT, especially under aerobic conditions. The following sequence of events illustrated in figure VII.3 was suggested:

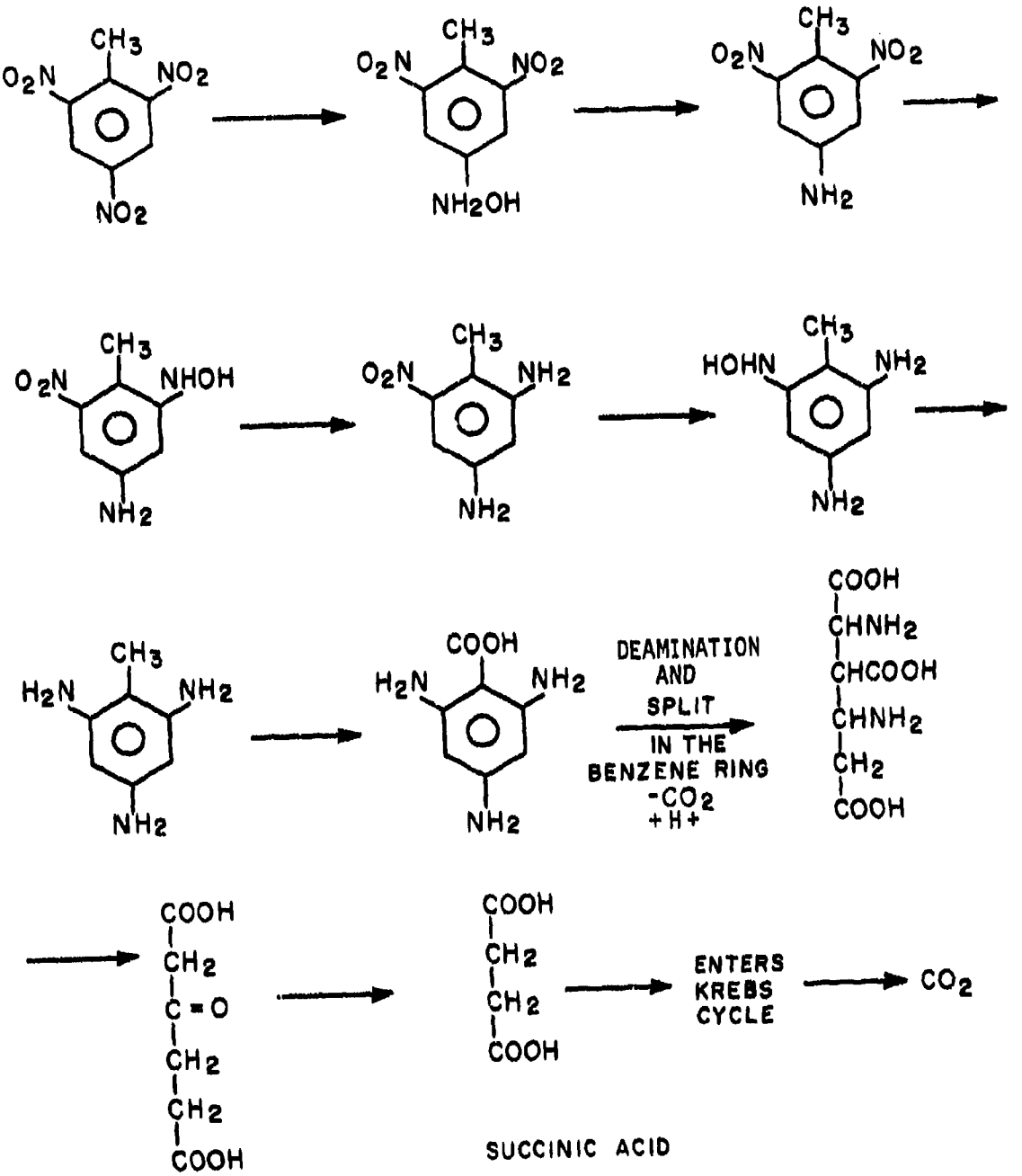


Fig. VII.3. BIODEGRADATION OF TNT IN *PSEUDOMONAS* SP.

VIII. EPIDEMIOLOGY

Before World War I, TNT was considered non-toxic. However, during World Wars I and II, several fatalities occurred among TNT workers, expecially during the first war; it was then looked upon as an unfortunate but inevitable risk of TNT production. The most common causes of death were toxic hepatitis and aplastic anemia. A number of case reports of intoxication and eventually death from TNT were mentioned in chapter III, under "Human Fatalities". The following is an enumeration of cases in chronological order.

1. In 1915, the first case of TNT intoxication occurred; by the end of that year, no less than 50 other cases of fat jaundice occurred among TNT workers (96, 97).

2. During World War I, although the production of TNT in the USA was on a comparatively small scale, 17,000 persons were intoxicated by TNT in a period of 7 1/2 months, of which 475 cases ended fatally. Hamilton (95) reported 95 cases of toxic jaundice due to TNT intoxication in 1917. In another report, Hegyeli (100) described 24,000 cases of TNT intoxication in the USA during the period from 1914 to 1918. Of these, 580 died.

In England 50 deaths due to toxic hepatitis during this war were reported.

3. During the same period only 1,000 cases of TNT intoxication were reported in Germany, of which 20 developed liver atrophy (100).

4. Fatalities due to TNT during World War II sharply decreased, due to the enforcement of strict hygienic measures; thus only 22 deaths were reported in the period from June 1941 to September 1945 (97).

In a detailed review of the occupational diseases in government-owned explosive plants in the period of February 1943 to April 1945, McConnell et al. (175) gave the following numbers for fatalities and diseases due to TNT exposure, among shell and bomb loading workers (Table VIII-1) and among workers in manufacturing works (Table VIII-2).

Several fatalities were also reported during World War II in England, Canada and Finland.

In Yugoslavia, 42 cases of TNT intoxication were reported in 1953 by Branisavljevic. Of these cases, 18 showed damaged liver, 19 suffered from anemia and 5 exhibited both damaged liver and anemia (176).

On a recent epidemiological study the relationship between hemoglobin values (Hb) and the extent of TNT exposures were reported by Hathaway (177). In this report, the effect was studied separately in males and females; whites and nonwhites. Results were as follows (Table VII-3).

In another study (178) examination of 43 TNT workers for hemoglobin level (Hb), serum glutamic oxaloacetic transaminase (SGOT), and lactic dehydrogenase (LDH) was performed, and followed for 5 months as the production of the plant was increased. The data in Table VIII-4 were reported.

TABLE VIII.1

FATALITIES AND DISEASES DUE TO TNT EXPOSURE IN WORKERS IN SHELL AND BOMB LOADING PLANTS

	Through Feb. 43	March 43 to May 43	June 43 to Aug. 43	Sept. 43 to Jan. 44	Feb. 44 to April 44	May 44 to July 44	Aug. 44 to Oct. 44	Nov. 44 to Jan. 45	Feb. 45 to April 45
Number of employees	14,700	15,700	17,400	15,000	16,200	15,700	19,600	22,000	21,100
Duration of exposure (months)	8.8	3	3	5	3	3	3	3	3
Number of deaths	3	2	3	6	1	0	2	1	0
Number of cases resulting in loss of time									
- systemic poisoning	89	63	89	41	6	14	13	7	18
- dermatitis	48	51	22	37	19	26	26	27	14
Total	137	114	111	78	25	40	39	34	32
Number of cases of mild disease									
- systemic effects	1,467	1,470	2,760	2,073	899	591	740	390	822
- dermatitis	8,711	2,925	2,411	3,102	1,175	1,443	1,543	1,077	901
Total	10,178	4,395	5,171	5,175	2,074	2,034	2,283	1,467	1,723
Number of medical transfers									
- systemic effects	not reported	1,125	1,836	1,255	293	407	328	392	751
- dermatitis	not reported	507	615	915	450	676	687	369	122
Total	——	1,632	2,451	2,170	743	1,083	1,015	761	873

Ref.: McConnell et al. (175).

TABLE VIII.2
FATALITIES AND DISEASES DUE TO TNT EXPOSURE IN WORKERS IN MANUFACTURING PLANTS

	Through Feb. 1943	March 43 to May 43	June 43 to Aug. 43	Sept. 43 to Jan. 44	Feb. 44 to April 44	May 44 to July 44	Aug. 44 to Oct. 44	Nov. 44 to Jan. 45	Feb. 45 to April 45
Number of employees exposed to TNT	3,600	5,000	5,500	6,900	4,200	5,700	6,100	7,600	8,200
Duration of exposure (months)	11	3	3	5	3	3	3	3	3
Number of deaths	1	0	0	0	0	0	0	0	0
Number of cases resulting in loss of time									
- systemic poisoning	13	6	9	4	2	0	2	0	3
- dermatitis	29	0	5	3	1	0	1	0	3
Total	42	6	14	7	3	0	3	0	6
Number of cases of mild disease									
- systemic effects	168	219	313	262	125	78	48	29	28
- dermatitis	1,980	834	990	586	180	331	337	265	297
Total	2,148	1,053	1,303	848	305	409	385	294	325
Number of medical transfers									
- systemic effects	not reported	210	207	225	94	55	51	31	40
- dermatitis	not reported	105	144	95	46	69	59	48	74
Total	——	315	351	320	140	129	110	79	114

Ref.: McConnell et al. (175).

TABLE VIII.3
RELATIONSHIP BETWEEN HEMOGLOBIN VALUES
AND EXTENT OF EXPOSURE TO TNT

Exposure levels mg/m^3	Whites				Nonwhites			
	Mean Hb[a]		Relative Odds		Mean Hb[a]		Relative Odds	
	M	F	M	F	M	F	M	F
Not exposed	15.2	13.7	1.0	1.0	14.7	13.1	1.0	1.0
0.01 or less	15.0	13.6	1.1	1.4	14.7	12.9	0.8	1.5
0.02 to 0.09	14.7	13.6	1.8	7.0	14.9	13.3	0.5	0.0
0.10 to 0.19	14.7	13.5	1.9	0.0	14.3	12.9	2.1	2.5
0.20 to 0.29	14.4	13.8	2.8	0.0	13.9	--	1.2	--
0.30 to 0.39	14.0	10.6	6.9	--	13.7	--	2.5	--
0.40 to 0.49	14.8	13.6	2.9	0.0	13.8	12.8	3.5	3.8
0.50 to 0.99	14.4	13.4	6.0	8.0	13.6	13.0	7.5	--
1.00 to 1.49	13.7	--	6.2	--	--	--	--	--
1.50 and over	--	12.4	--	--	13.1	--	--	--

a. The following numbers for Hb values were considered abnormal:
Males <14 g/100 ml blood;
Females <12g/100 ml blood.

b. Relative odds (R.O.) is a relative number that was calculated as follows:

$$R.O. = \frac{\text{Number of control cases showing normal values X number of exposed cases showing abnormal values}}{\text{Number of control cases showing abnormal values X number of exposed cases showing normal values}}$$

Ref.: Hathaway (177).

TABLE VIII.4
HEMOGLOBIN, SERUM GLUTAMIC OXALACETIC TRANSAMINASE AND LACTIC DEHYDROGENASE OF WORKERS BEFORE AND AFTER EXPOSURE TO TNT

Test	Pre-employment	Dec. 1973	Jan.	Feb.	Mar.	Apr. 1974
Hb	15.6[a]	15.0	15.2	15.1	14.8	14.9
SGOT	35	35	32	39	58	54
LDH	64	53	52	51	106	103
Production rate (% capacity)	--	75	75	75	126	100
Average TNT level (mg/m^3)	--	0.3	0.3	0.3	0.8	0.6

a. Abnormal values were considered as follows: Hb <13.5; SGOT >45; LDH >100.

Ref.: Morton and Ranadive (178).

Demographic distribution of these cases was studied according to sex, ethnic and age groups; results were as follows (178).

	Total no. examined	% of Abnormal Values	χ^2	P
Sex:				
Male	37	56.8	0.21	0.7>p>0.5
Female	6	100	1.32	0.3>p>0.2
Ethnic group				
Caucasian	42	61.9	0.01	0.95>p>0.90
Black	1	100	0.22	0.70>p>0.50
Age group				
18 to 29	29	55.2	0.27	0.7>p>0.5
30 to 39	7	71.4	0.08	0.8>p>0.7
40 to 49	3	100	0.66	0.5>p>0.3
50 and over	2	100	0.44	0.7>p>0.5
Unknown	2	50	0.22	0.7>p>0.5

In order to detect the toxic effects of TNT by biochemical changes, Morton and Ranadive (178) carried out epidemiological studies on 27 cases to determine which one of these tests (Hb, SGOT, LDH) might be used for routine monitoring of cases. Their results were as follows:

Test or Combination	Number of Abnormals Detected	% of Total Abnormals Detected
Hb	22	25.9
SGOT	41	48.2
LDH	50	58.9
Hb and SGOT	57	67.1
Hb and LDH	69	81.2
SGOT and LDH	71	83.5
SGOT, LDH and Hb	85	100.0

They concluded that none of the above-mentioned single tests was sufficiently sensitive to be used as the only indicator for the detection of TNT toxicity. As they indicated, all three tests should be carried out simultaneously to detect the toxicity from TNT.

IX. INDUSTRIAL HEALTH HAZARDS, HYGIENIC AND SAFETY MEASURES AND STANDARDS FOR TNT

The potential health hazards associated with the production and handling of TNT constitute a constant threat that should not be overlooked, especially in the light of the fact that an estimated 200,000 tons of TNT were produced in U.S. Army Ammunition plants in 1973 (179). It is well known that the several cases of intoxication and fatalities that occurred during World War I, seriously hampered efficiency and production of TNT, and disturbed the morale of employees to the point that the manpower problem was markedly aggravated. However, by enforcing strict safety and hygienic measures, the number of fatalities from TNT during World War II was greatly reduced.

This chapter is divided into 3 main sections showing the industrial health hazards encountered during the manufacture and handling of TNT; various safety and hygiene measures to be undertaken; and a presentation of standards for exposure to this explosive.

1. Industrial Health Hazards

TNT is one of the most widely used high explosives on account of being comparatively safe in manufacture and storage. However, if extreme care is not exercised in dealing with TNT, explosion and health hazards might occur.

A. Explosion and Fire Hazards

Although TNT is one of the least sensitive explosives to impact and friction, the presence of gritty foreign materials render TNT much more sensitive to impact. Also, it can be detonated by moderate force when confined between metal surfaces such as on threads of bolts; by burning thick pieces in cast form, and by strong shock (2 kg falling for 130 cm was necessary to induce detonation) or sudden and rapid heating (2, 7, 180).

TNT does not appear to be reactive in contact with acids; however, it reacts vigorously with alkalies and reducing agents(see Chapter II). TNT should not be used in the presence of heavy metals or admixed with large amounts of activated carbon (2).

B. Disaster Hazards

TNT emits toxic fumes of nitrogen oxides on decomposition (8).

C. Health Hazards

The effects in workers produced by TNT exposure were discussed in detail in Chapter III. The following is an enumeration of health hazards that can be caused by acute or chronic exposure to TNT, based upon that discussion:

1. Liver: acute yellow atrophy and toxic jaundice

2. Blood: Methemoglobinemia with the consequences of oxygen deficiency, hemolytic anemia (in G6PD-deficient workers), aplastic anemia, and thrombocytopenia

3. Heart: depression of the myocardium; various electrocardiographic changes

4. Blood vessels: increased permeability to proteins

5. Pancreas: exocrine dysfunction

6. Skin: dermatitis, eczema

7. Eye: arc-shaped cataract in the lens after forming an irregular ring.

8. Teeth and oral cavity: caries and stomatitis

9. Central nervous system: various functional changes

10. Kidney: partial retention of sodium, increased excretion of porphyrins

11. Biochemical changes: increase in plasma bilirubin and possible changes in SGOT, SGPT, and SDH.

12. Gastrointestinal tract: gastritis, nausea and vomiting; epigastric pain

D. Toxic Hazard Rating: (8)

1. Acute local: irritant 2*

2. Acute systemic: ingestion 3**; inhalation 3; skin absorption 2.

3. Chronic local: irritant 2; allergen 2

4. Chronic systemic: ingestion 3; inhalation 3; skin absorption 3.

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* 2 = Moderate, may involve both irreversible and reversible changes not severe enough to cause death or permanent injury.

**3 = High, may cause death or permanent injury after very short exposure to small quantities.

2. Hygienic and Safety Measures

Measures taken to protect the health of TNT workers have proved successful. These measures can be subdivided into: personnel measures and safety regulations, chemical and engineering, and medical measures.

A. Personnel Measures and Safety Regulations

To many people, engineering and medical measures taken to protect TNT workers are enough to decrease the number of TNT exposures in munition plants. However, the employment of careless persons would end in the occurrence of intoxication, no matter how well other measures are followed. It is, therefore of great importance to select the proper workers who can not only understand the danger of TNT to their health, but who also comply with the various safety regulations existing in TNT plants. Personnel and Safety measures can be grouped under the following main headings (18,20,24,27,28):

i. Selection of Workers

The following measures were taken in selecting employees for TNT workers:

1. No person below 18 years of age should be employed in TNT manufacture at any stage of production.

2. No one showing signs of anemia, having a history of gastric troubles or dyspepsia, chronic alcoholism or asthma should be admitted.

3. Personal cleanliness is of great importance; those who show signs of poor personal hygiene practices should not be employed.

4. Differences in susceptibility to TNT intoxication due to race and sex are disputed. Women and caucasians have sometimes been found to be more susceptible than men and black people.

ii. Personal and Workshop Cleanliness

Strict personal cleanliness of workers and all personnel handling TNT is mandatory. Special attention should be paid to frequent cleaning of skin, hair, teeth, and fingernails. Throat and mouth wash may be supplied to workers.

Furthermore, cleanliness of workshops provides the first line of defense against exposure to TNT. This can be attained by the use of local exhaust ventilation, dusting with dampened cloths and sweeping floors with dampened brooms.

iii. Educational Programs

All workers ought to be taught by the medical officer to look upon TNT as a dangerous substance that can cause serious disease and eventually death. It should be emphasized that TNT can be absorbed not only via skin but can also be inhaled. Notices should be placed in conspicuous places in all shops, stating the dangers and precautions to be observed. Regulations should be strictly observed and enforced.

iv. Alternation of Workers

In the past, it was the plan of some plants, as a preventive measure against TNT poisoning, to alternate workers on a weekly or fortnightly basis. However, this has proven to be an inefficient way of preventing toxicity.

v. Handling and Storage

TNT should be handled with care, by people wearing mechanical filter respirators, rubber gloves and protective overalls. It should be stored in a permanent magazine, away from sources of heat and initiator explosives. TNT should also be kept separate from oxidizing and combustible materials (7). Storage quantities and distances should conform to the Americal Table of Distances for Storage of Explosives (2).

vi. Shipping Regulations

Details of safety measures for shipping of TNT are mentioned in the Ordnance Safety Manual ORD M7-224 published by the Ordnance Corps, Department of the Army (2).

B. Chemical and Engineering Measures

From Chapter VII it is clear that TNT can gain access to the body through inhalation, gastrointestinal tract and/or skin. Protective measures should be taken, therefore, to prevent the concentration of TNT fumes or dust from exceeding the Occupational Safety and Health Administration Standard. Continuous monitoring of TNT in air is mandatory. Furthermore, clean protective garments should be provided for workers to guard against skin absorption of TNT. Recommended measures (28) include:

i. Determination of TNT in Air

The level of TNT in air should not exceed the present OSHA standard of 1.5 mg/m^3. Methods for determination of TNT in air are mentioned in the following chapter.

ii. Temperature and Humidity Control

Dermatitis from TNT is exacerbated by hot weather (see Chapter III). Therefore, control of temperature, humidity and air motion in manufacturing areas is crucial.

iii. Proper Ventilation

The degree of exposure to TNT at various stages of production and handling as well as the measures necessary to prevent excessive exposures are compiled by Brandt (81) in Table IX.1. Local exhaust ventilation is recommended to keep worker exposure at safe levels of TNT in workroom air.

iv. Disposal and Waste Treatment

Apart from other techniques such as evaporating TNT waste or draining it into the the nearest stream, or the methods which are discussed in detail in Chapter VII, the following can be used (7).

TABLE IX,1

IMPORTANT ATMOSPHERIC HEALTH HAZARDS AND
RECOMMENDED CONTROL MEASURES BY TYPE OF ESTABLISHMENT

Operation	Nature of Exposure	Degree of Exposure	Recommended Control Measures
Screening	TNT dust. Infrequent to continuous.	Severe	Enclose operations. Provide local exhaust ventilation at minimum rate of Q = 300 A. Respirators needed if control is not adequate. (Q represents rate of air removal in cubic feet per minute). (A represents effective area of hood opening in square feet).*
Melting	TNT dust, fume and vapor. Intermittent. Few workers.	Slight	Provide exhaust ventilation at minimum rate of Q = 150A. (A represents area of one lid or door).
Draw off	TNT fume and vapor. Few workers direct, many indirect.	Moderate but usually intermittent.	For infrequent operations - Respirators. For frequent operations - Room ventilation and respirators or local exhaust ventilation - Q about 200 c.f.m.
TNT cooling	TNT fume and vapor. Few to many workers. Intermittent to continuous	Slight to severe.	Mechanical agitation. Respirators for infrequent exposures. Adequate room ventilation and respirators, or preferably local exhaust ventilation at kettles (Q = 120A for continuous exposures). (A represents area of top of kettle).
Pouring	TNT dust, fume and vapor. Many workers.	Slight to moderate	Careful filling and puddling. Elimination of all unnecessary sources of atmospheric contamination. Good general ventilation. Rotation of workers. Respirators.

TABLE IX.1 (Cont.)
IMPORTANT ATMOSPHERIC HEALTH HAZARDS AND
RECOMMENDED CONTROL MEASURES BY TYPE OF ESTABLISHMENT

Operation	Nature of Exposure	Degree of exposure	Recommended Control Measures
Bomb filling (Draw off)	TNT fume and vapor. Few workers. Intermittent.	Slight to severe.	Have valve operator stand to side of bomb and remove fumes from room. Local exhaust ventilation - Q about 200 c.f.m. per draw off site.
Bomb puddling; nose and tail pouring	TNT dust, fume and vapor. Many workers. Intermittent to continuous.	Slight to moderate	Same as pouring.
Adding TNT scrap	Same as above.	Moderate to severe	Careful handling of scrap. Good general ventilation. Clean gloves. Rotation of workers. Respirators.
Booster cavity drilling or reaming and thread cleaning	TNT dust. Few to many workers. Intermittent to continuous.	Slight to severe	Eliminate deep drilling. Apply suction during entire cycle, or local exhaust ventilation.
Riser knock off	TNT dust. Few to many workers. Intermittent to continuous.	Slight to moderate	Employ risers of good design. Use care in knocking risers off. Isolate and exhaust ventilate if possible.
Riser break out	TNT dust. Few direct, many indirect. Usually intermittent.	Moderate to severe	Exhaust hood or booth similar to spray paint booth. Ventilation rate - Q = 300 A.

TABLE IX.1 (Cont.)
IMPORTANT ATMOSPHERIC HEALTH HAZARDS AND
RECOMMENDED CONTROL MEASURES BY TYPE OF ESTABLISHMENT

Operation	Nature of Exposure	Degree of Exposure	Recommended Control Measures
TNT scrap Break up	TNT dust. Few direct, many indirect. Essentially continuous.	Moderate to severe	Different method of scrap production, or exhaust hood or booth similar to spray paint booth. Ventilation rate - Q = 300A
Tubs and buckets break out	TNT dust. Few direct, many indirect. Intermittent.	Moderate to severe	Exhaust hood or booth similar to spray paint brush. Ventilation rate - Q = 300A - or isolate operation and have workers wear respirators.
Flaking or graining	TNT fume and dust.	Insignificant to moderate	Enclose process as much as possible and exhaust ventilate at minimum rate of Q = 150A.
Box filling	TNT dust. Few workers.	Slight to severe.	Provide local exhaust ventilation at source of dust through hood formed of shrouded flange at end of chute. Q = 200A.
Box shaking	TNT dust.	Slight to severe	Provide local exhaust ventilation at source of dust through hood of same shape as for box filling. Minimum ventilation rate Q = 200A.

*Q = Rate of Flow

Ref: Brandt (181).

1. Dissolve TNT in alcohol, benzene or any combustible solvent, then spray the solution into a furnace with afterburner and scrubber.

2. TNT can be treated with sodium bicarbonate or mixed with sand-soda ash (9:1) and then burned in an open furnace.

C. Medical Measures

To perform medical examination of TNT workers, the following facilities should be available: dispensary, laboratory, first aid stations, ambulance service and a well equipped plant (and community) hospital. Furthermore, it is of paramount importance to have plans and procedures for handling large number of casualties in the event of a disaster.

The important role played by medical personnel does not end by performing pre-employment physical examinations (see part A.), but rather is continuing. The following are the responsibility of the medical team:

i. Routine Check on All Employees

Evaluation of liver function and hematologic status, as well as physical examination of all employees should be carried out routinely. Workers with early symptoms of jaundice, skin pallor, cyanosis or dermatitis should not be allowed to continue work (28). Complete blood counts and liver function tests are also recommended every 6 months (7). Special recheck examination for those in toxic exposures (175) as well as supervision of TNT absentees (182) are recommended.

ii. Sanitary Supervision

Medical personnel are responsible for medical inspection of all operations, control of food and water sanitation, sanitation of bathrooms and bathhouses, and control of workers' personal hygiene. The following hygienic facilities should be provided.

1. Since TNT can be absorbed through the skin, daily showers are important. Ordinary soap is not effective in completely removing TNT; therefore a special liquid soap to which is added 5 to 10% potassium sulfite and 5 to 15% of an appropriate wetting agent, can be used (28).

2. A clean work suit, cap and underwear should be provided to each employee every day (183,184) to prevent skin contact. This protective clothing should be designed to minimize skin contact at the neck, wrists and ankles. Eye contact can be prevented by using safety glasses or face shields (2). To prevent ingestion of TNT, food and drink must be excluded from the work area. [Nutritious meals are, however, recommended (182).]

3. Standards for TNT

The threshold limit value (TLV) of 1.5 mg/m^3 for TNT was adopted as a standard by the U.S. Occupational Health and Safety Administration (185). The safety of this TLV value is questioned by several authors (100, 177, 179). The maximum allowable concentration of TNT in the U.S.S.R. and Czechoslovakia is 1.0 mg/m^3. Buck and Wilson (179) recommend a TLV of 0.5 mg/m^3 for atmospheric TNT. The U.S. Army has lowered its standard to 0.5 mg/m^3 as specified in DARCON Regulation 40-3, 10 November 1976.

X. SAMPLING AND ANALYSIS OF TNT

TNT is commercially prepared by the three-stage nitration of toluene, a process during which various trinitro-isomers are produced (see chapter II). It can also be prepared by a continuous process in which toluene is nitrated to the mono-, di-, and trinitro products in a series of eight nitration vessels (186). Purification of TNT is accomplished by washing crude TNT with aqueous sodium sulfite solution (16%) which is strongly nucleophilic towards activated aromatic nitro groups. All nitro groups in α-TNT being meta to one another are not activated to any extent. However, in all other isomers (mentioned in Chapter II) there is one nitro group that is ortho to a second nitro group and ortho- or para- to a third one. This results in a double activation of the first group by a second and third one; thus the 5-nitro of TNT, and the 3-nitros of β- and η-TNT (the major isomeric impurities which amount to 3 to 5%) are readily displaced by sulfite ion, which results in the formation of the water-soluble dinitrotoluenesulfonates. The intensely colored wash solution, known as "red water", which contains 25% dissolved solids (17) is usually drained to the nearest water stream. When such TNT-containing water is exposed to sunlight, it develops a highly visible color and is called "pink water".

Rapid analytical methods are then necessary to determine the crude and refined TNT, during the manufacturing process, as well as to detect the extent of environmental pollution in "pink water" which might prove fatal to aquatic creatures and to man.

Furthermore, detection and identification of trace amounts of TNT in air, particularly in the atmosphere of munition plants is of paramount importance to keep the concentration of TNT below the OSHA standard of 1.5 mg/m^3. Furthermore, as airplane bombings, hijackings and various threats to civilian areas, such as banks and public buildings, have grown in intensity and sophistication, the need for devices to detect ultra-trace quantities of TNT has become crucial.

Likewise, methods to detect TNT in the biological fluids of workers and experimental animals exposed to this explosive are of extreme importance.

Methods of sampling and analysis of TNT are mentioned hereafter according to the purpose for which they are used. Thus, the following topics will be discussed:

1. Analysis of TNT during manufacturing
2. Analysis of TNT in water
3. Analysis of TNT in biological fluids:
   - A. Urine
   - B. Blood
4. Analysis of TNT in air.

1. ANALYSIS OF TNT DURING MANUFACTURING

Several methods for the determination of the purity of TNT during the process of nitration and subsequent purification are employed. Among these are paper chromatography, thin layer chromatography, gas chromatography, potentiometric and colorimetric methods.

A. Paper Chromatography

Rapid identification of TNT can be achieved by one-dimensional descending paper chromatography using 2 different systems: a.) formamide (25% in acetone for impregnation) as stationary phase and 1:1 cyclohexane-benzene as mobile phase; b.) heavy mineral oil (10% in n-hexane) with ion exchange water. In both systems TNT has an Rf of 5 to 6. The Rf values for other nitro compounds have been obtained (187).

B. Thin Layer Chromatography (TLC)

In order to study and improve the continuous TNT process, as well as to detect the impurities during the process of purification, Kohlbeck et al. (188) mentioned the details of a thin-layer chromatographic separation of TNT from other impurities. Quantitative determinations using TLC are quite difficult due to variations in spot shapes and sizes, staining reagents and densitometric response. This fact, along with the low volatility of TNT, motivated Doali and Juhasz (189) to use high speed liquid chromatography, which provides favorable speed, and yields a good quantitative analysis in the microgram range.

Common impurities and some products of TNT oxidation or reduction can be separated and identified by two-dimensional thin-layer chromatography (TLC), as described by Yasuda in 1964 (190).

Possible separation of mixtures of isomers of TNT and byproducts from the production of di- and trinitrotoluenes was accomplished by the use of thin-layer chromatography (TLC) on silica gel G and ethyl acetate-petroleum ether as solvent (191).

C. Gas Chromatography (GC)

An accurate gas chromotographic technique for the determination of TNT, its isomers, and other impurities in samples taken throughout the continuous TNT manufacturing process, was described by Dalton in 1970 (192). This method depends on the use of a flame ionization detector which can be programmed to obtain adequate separation of the various components when the sample contains aromatics with all degrees of nitration.

Separation and identification of TNT and its isomers (except for 2,3,6-TNT) as well as DNT isomers that might be present in a sample of TNT, was carried out by Gehring and Shirk (193) using gas chromatography.

D. Potentiometric Methods

A non-aqueous potentiometric titration of TNT as acid was described by Sarson in 1958 (194). TNT, dinitrotoluene (DNT) and mononitrotoluene (MNT) are differentially resolved by titration in methyl isobutyl ketone and dimethylformamide.

Another potentiometric method for the determination of TNT in non-aqueous media in the presence of nitric and sulfuric acid, was mentioned by Aksenenko and Tatarnikova (195).

E. Colorimetric Methods

TNT can be determined indirectly during the manufacturing process by determining the undesired TNT isomer, 2,4,5-TNT, using a colorimetric method (196). This method detects changes in 2,4,5-TNT content indicative of malfunction in the TNT process.

2. ANALYSIS OF TNT IN WATER (TNT WASTES)

Colorimetric methods as well as methods utilizing gas and liquid chromatography have been described.

A. Colorimetric Methods

A simple method for the determination of TNT in TNT wastes was described in 1968 by Mudri (197). This depends on the color that develops by the action of sodium sulfite and sodium hydroxide solutions on TNT, which can be determined colorimetrically or spectrophotometrically at a wavelength of 500 nm.

Hess et al. (198) reported a colorimetric method for the determination of TNT in water. The method depends on treating the water sample with 15% potassium hydroxide solution to produce a Meisenheimer complex, whose intensity can be monitored at 440 nm using an analytical calibration curve established from known solutions of TNT. This method is usable to 80 ppm α-TNT and the analytical working curve is linear up to 20 ppm.

B. Gas Chromatography

Obsolete TNT has been disposed of in deep sea water. It is possible that sea water near these dumping areas can be polluted by TNT and that is why Hoffsommer and Rosen (199) reported in 1972 a method whereby parts per billion to parts per trillion of TNT in sea water can be detected. The details of this method which implies the use of vapor gas chromatography with the nickel-63 electron capture detector, are described. Another method for analysis of TNT in sea water and ocean floor sediment was described by the same authors (200).

C. Liquid Chromatography

Characterization of TNT in waste waters by liquid chromatography was described by Walsh et al.(201). Adsorption of nitro compounds was accomplished on an adsorbent resin of the styrene-divinylbenzene copolymer type.

3. ANALYSIS OF TNT IN BIOLOGICAL FLUIDS

Determination of TNT in biological fluids is of great importance to detect the amount of TNT in victims of TNT intoxication. Methods for determination of TNT in urine and blood are presented.

A. Urine Analysis

The following tests were used to detect TNT in the urine of persons and experimental animals that were exposed to TNT.

i. Webster Test (1916)

This test, although commonly used for detecting TNT in urine, is only a qualitative indication of exposure to TNT; and is considered by several authors to be an unreliable method of detecting early TNT intoxication (Snyder and von Oettingen 1943, 202; Pinto and Wilson 1943, 203). This test was introduced by Webster in 1916 (204), and depends on the formation of purple color where the acidified urine samples are extracted with ether, and the latter is treated with an alcoholic solution of potassium hydroxide. This test is undependable because the color developed is "fugitive" and might give rise to erroneous conclusions; furthermore, negative results were obtained in severe cases of TNT intoxication.

Three years later, Elvove, in 1919 (205) tried to modify the Webster test and make it "nearly quantitative", but the need for a more specific and quantitative test for TNT was necessary; the following methods were, therefore, adopted.

ii. Method of Snyder and von Oettingen (1943)

This method depends on the presence of the TNT metabolite, 2,6-dinitro-4-aminotoluene in the urine of men and experimental animals exposed to TNT. The amine is extracted from urine by ether, then after evaporation of the ether, the residue is diazotized and coupled with α-naphthylamine. The stable orange yellow color (C. F. Webster test) of a toluene extract is then compared with a set of standards. Using this method to analyze the urine of 98 women and 245 men exposed to TNT the average daily excretion of the amine was 1.29 mg and 2.06 mg, respectively (202).

In a recent article, however, Hassman (206) found a close correlation between the Webster reaction and the ascertained values of 2,6-dinitro-4-amino-toluene in the urine of 55 workers.

iii. The Method of Pinto and Wilson (1943)

This is a photoelectric colorimetric method and basically depends on the diazotization and coupling with dimethyl-α-naphthylamine of the triaminotoluene. The latter is produced from TNT and its derivatives that are extracted from urine by ether, and subsequently reduced, in aqueous solution, by titanium chloride. This method does not differentiate between TNT and other aromatic nitro compounds present in urine, and great variations in results were obtained. Expressed as TNT, the amount of TNT derivatives in the urine of workers exposed to TNT varied from 1 to 101 µg/ml of the urine (203).

B. Blood Analysis

Roubal in 1949 (207) described the use of a polarographic method for the determination of TNT in blood. No characteristic polarographic wave could be demonstrated in the erythrocytes after intravenous injection of 20 mg of TNT to dogs. However, TNT could be detected in erythrocytes and plasma of a 5.3 kg dog that was given 0.25 g TNT orally, only after allowing the reduction solution (metol-hydroquinone) used for the determination to act for a considerable period of time.

A photocolorimetric method for the estimation of 2,6-dinitro-4-aminotoluene, a product of TNT biotransformation, in blood of exposed workers, was described by Chiecchio in 1960 (208).

4. ANALYSIS OF TNT IN AIR

Rapid detection of trace amounts of TNT in ambient atmosphere is necessary not only for monitoring its concentration at various stages of production in ammunition plants, but also to give the most viable approach for the detection of hidden explosives. The concentration of TNT in air, directly above the sample, at room temperature is 10^{-5} mg/l (209).

A number of laboratory procedures for the detection and identification of TNT are available, among which is gas chromatography (GC) with electron capture detectors (ECD), mass spectrometry (MS) or GC-MS. The drawbacks of these methods are: a.) generally they involve a preconcentration step from a large air sample; b.) usually there is a considerable loss of sample through surface adsorption, a phenomenon which is very significant in the case of TNT; and c.) lack of selectivity. These disadvantages can be overcome by the use of the modern technique: plasma chromatography.

A. Spectrophotometric Methods

TNT dust and vapors can be measured simply by passing the air sample through a midget impinger containing diethylaminoethanol; a reddish coloration that is proportional to the TNT content develops and can be measured spectrophotometrically at 490 nm (210).

B. Colorimetric Methods

Determination of TNT in the workroom atmosphere was carried out by Kay (211). The method which depends on the red coloration produced when TNT in acetone is treated with an aqueous solution of sodium hydroxide, is not very accurate (± 10%).

Another colorimetric method for the estimation of airborne TNT was mentioned a few years later by Cumming and Wright (212). This depends on the absorption of TNT in a mixed solvent of methyl ethyl ketone and cyclohexanone, to which a little potassium hydroxide solution was added. A special Tintometer disc was used for comparison.

A colorimetric method for the determination of TNT in air, in the presence of DNT was described by Gronsberg in 1962 (213). The method depends on collecting TNT in air (which must not exceed 0.1 mg) on an ashless filter, which is then washed with alcohol. The alcoholic solution is then treated with sodium hydroxide solution, and the violet color thus obtained is compared with a series of standards.

C. Gas Chromatography (GC) Techniques

Gas chromatography offers a reliable means of separating and estimating the individual TNT isomers as well as other dinitrotoluenes and other impurities

that might be present. Furthermore, it provides the advantage of the possibility of the development of an automated instrument for the continuous monitoring of atmospheric TNT. The proper choice of the GC column provides the means by which the GC technique can be selective for TNT. The use of the proper sampling techniques and of an electron capture detector can increase the sensitivity to traces of TNT, which has strong electron-capturing characteristics.

A method for the qualitative and quantitative detection of vapors of TNT which depends on the use of an electron capture detector was invented in 1975 by Jenkins (214).

An analytical method for evaluation of exposures to TNT in the work place was described by Saltzman et al. (215). Samples are collected at the breathing zone of the worker on organic-free glass fiber filters. The TNT thus collected is dissolved in ethyl acetate and injected into a portable gas chromatograph equipped with a flame ionization detector. Methods of generation of trace vapor concentrations of TNT for calibrating exposive vapor detectors were mentioned by Pella in 1976 (216).

The main problems encountered in the use of automated GC apparatus can be enumerated as follows:

1. The low resolution and peak tailing of interfering substances can obscure results.

2. The significant effect of previous TNT samples, other impurities and materials from the sample inlet surfaces on the memory of the sample concentration system.

3. A very close control of the column temperature and carrier flow is mandatory.

D. Mass Spectrometry (MS)

Compared to GC, MS offers the advantages of selectivity, rapid response and high sensitivity for the detection of traces of TNT in air. The mass spectra of military grade TNT (which is nearly pure α-TNT with small amounts of other isomers as well as several isomers of dinitrotoluene) was reported by Murrmann et al. (217) in 1971. The mass spectra obtained revealed that each compound has a strong, distinctive base peak M^+ at m/e 17. Also, the abundant fragment ion at m/e 63 and 89 permits measuring the sum of these two compounds (TNT and 2-4 DNT) that are usually present together in explosives. Results of analysis of a typical military grade of TNT are as follows:

TABLE X.1

Composition of the Solid and the Equilibrium Vapor Phases of Typical Military Grade TNT

Compound	Solid phase composition (%)	Vapor phase composition (%)
2,4,6 TNT	99.80	58(1×10^{-5} mm)
2,3,5 TNT	0.08	trace
2,3,4 TNT	0.02	3
2,4 DNT	0.08	35
2,5 DNT	<0.01	4
3,5 DNT	<0.01	trace
3,4 DNT	trace	trace
2,6 DNT	trace	trace
Other impurities	None detected	No determination

Ref.: Murrmann et al. (217).

A totally automated quadrupole mass spectrometer specifically designed by Olfax Instruments and computerized for the routine monitoring of TNT was evaluated by Spangler (218). In this instrument a gas or liquid sample can be directly introduced through a silicone membrane interface. In his report, Spangler performed a careful theoretical analysis of the permeability of TNT to the dual stage silicone membrane interface, and followed this section with experimental observation. He found that the sensitivity of the Olfax Monitor to TNT vapor is 1 part in 10^{10} at m/e 89, and 1 part in 10^{9} at m/e 210. He also found that the optimum temperature for the membrane inlet system in case of TNT is 150 to 160°C, at which temperature losses of vapor transport due to adsorption are minimized.

One of the major drawbacks in the use of mass spectrometry is that oxygen and water present in the air sample are admitted through the membrane to the MS in source. These compounds proved to have an unfavorable effect on the ionizing filament and also deposited an insulating film on the MS surfaces (209).

E. Plasma Chromatography

Plasma chromatography provides a simple and rapid method that is sufficiently selective and sensitive to detect TNT in ultra-trace concentrations in air. The principle of this method is the use of an ion-molecule reactor at atmospheric pressure, coupled to an ion-drift spectrometer to produce characteristic ion mobility spectra of trace compounds contained in the carrier gas. Using this technique Wernlund (219) was able to detect selectively nanogram and less quantities of TNT in river water. Karasek (209) and Karasek and Denney (219) were able to identify picogram or less quantities of TNT in air. TNT produced strong positive and negative mobility spectra; using the plasma chromatograph, a rapid analysis of TNT in air can thus be obtained.

Extremely low concentrations of TNT in air can be detected by utilizing an electron transfer reaction in combination with a low resolution mass filter (220). This method depends on mixing the air sample under investigation with a negatively ionized gas in a reaction chamber. Electrons are transferred from the gas to TNT molecules to form negative ions. A drift field is then applied to the mixture in the reaction chamber to transport the negative ions therein in a desired direction out of the reaction chamber. The ions thus transported generate an electrical current, which provides an indication of the TNT concentration in air. Mixtures of TNT and nitroglycerin can also be detected.

In an exhaustive survey of the sensitivity and specificity of several detection systems used for the determination of explosives in air, Wall and Gage (221) examined the following methods:

1. Ion mobility spectrometer (a type of plasma chromatograph)
2. Bioluminescent sensor system
3. Portable quadrupole mass spectrometer
4. Model 27 Gelignite detector
5. Model 58 explosive detector
6. Explosive detection dogs.

Concerning TNT, the following results were given.

Detection System	Specificity	Sensitivity
Bioluminescent Sensor System	Fair	30 PPB*
Mass Spectrometer	Excellent	25 PPB
Model 58, Explosive Detector	Fair to Good	0.2 PPB

* Parts Per Billion

XI. TECHNICAL SUMMARY

The compound 2,4,6-trinitrotoluene, known also as α-trinitrotoluene or simply TNT, was first synthesized by Wilbrand in 1863. Twenty-two years later, it was adopted by German military authorities for filling shells. Several other countries soon followed and millions of tons of TNT were used during World Wars I and II. It was estimated that 200,000 tons of TNT were produced in U.S. Army Ammunition plants in 1973 (179).

Before World War I, TNT was thought to be a safe and harmless compound, and was described by Dr. Prosser White, a then recognized authority on intoxication by the toluene derivatives, as an innocuous nontoxic substance which had never been known to produce illness. It was not until February 1915, that that prevailing belief was strongly shaken when the first death attributed to TNT and diagnosed as "toxic jaundice" was announced. However, in the manufacture of TNT, great care is taken against accidental explosions which are so great a threat that they usually overshadow the less spectacular but real danger of TNT exposure.

It is the objective of this monograph to review and evaluate the present status of knowledge of TNT and provide recommendations for future studies. For this reason, the following topics have been discussed: physical and chemical properties; human toxicity and fatalities; toxicologic investigations in animals; carcinogenicity, mutagenicity and teratogenicity; relationship between dietary factors and TNT poisoning; absorption, distribution, biotransformation, excretion and biodegradation; epidemiology; industrial health hazards, hygienic and safety measures and standards; and sampling and analysis of TNT.

1. PHYSICAL AND CHEMICAL PROPERTIES

TNT occurs as yellow monoclinic needles and melts at 80.9°C. In chapter II, its solubility in various solvents, its reactivity with different compounds, its stability and its sensitivity to impact were mentioned.

TNT can be prepared by nitration of toluene by the three-stage or by continuous processes. Crude TNT thus obtained contains 5 to 7% impurities; the chief contaminants are β- and γ-isomers (see Figure II.1.), and also traces of trinitrobenzoic acid, trinotrobenzene and tetranitromethane. These impurities not only decrease the melting point of TNT but also impart an objectionable greasy character. The presence of these impurities is also objectionable because the nitro groups in the ortho- and para- positions are labile in nature and are easily hydrolyzed with the liberation of free nitric acid. Munition grade TNT contains less than 1% impurities.

As an explosive TNT is about 95% as powerful as picric acid. The velocity of its detonation, which is a measure of violence of explosion, is about 7,000 meters a second; that of picric acid is 7,300.

TNT can be used as an explosive, alone or mixed with oxygen-rich substances such as ammonium nitrate. The power of 40/60 amatol (which contains 40% ammonium nitrate and 60% TNT) is 1.16; that of 80/20 amatol is 1.27 times that of picric acid.

TNT reacts with alkalies to form dangerously sensitive compounds; exposure to sunlight and ultraviolet radiation results in the formation of photodecomposition products (see Figure II.2.).

Commercial grade TNT is relatively safe in transport and storage as compared to other explosives. It is stable in contact with metal. TNT is reactive with aldehydes, alkalies, alkoxides, amines, acids, sulfides and sulfites (1,3).

2. HUMAN TOXICITY AND FATALITIES

Exposure to TNT is the main source of toxicity for workers in ordnance plants. However, exposure to the fumes in nitrating rooms, which consist of nitric acid fumes, nitrous oxide, methane, hydrogen and chlorine gases, can cause severe bronchitis and edema of the lungs. The human toxicity of TNT, on the other hand, will be briefly summarized according to its effect on various tissues.

A. ORGAN TOXICITY

a. Hepatotoxicity

Exposure of workers in ammunition plants to TNT during World War I resulted in 475 cases of toxic jaundice in England, of whom 125 died. In the U.S.A., 7,000 cases were reported and the death toll was 105 (23). During World War II, only 8 workers died from toxic hepatitis in the U.S.A. (24).

Toxic jaundice is due to the direct effect of TNT on the liver cells and apparently it follows degeneration and consequent obliteration of bile capillaries. The thymol turbidity test (23) as well as determination of SGOT and LDH in serum (177) were used to detect early liver impairment.

b. Hemotoxicity

Red Blood Cells (RBC): Exposure to TNT decreases the oxygen carrying capacity of RBC by the formation of methemoglobin (37) and nitric oxide hemoglobin (36). A decrease in number and hemoglobin content of RBC was also reported after exposure to TNT. Cases of aplastic anemia, which together with toxic hepatitis are the main causes of death in TNT toxicity, have been reported (31, 34, 43, 44). Some believe that TNT-induced aplastic anemia is a rare response occurring only in certain susceptible persons where the bone marrow is depressed regardless of age or physical characteristics (42).

Aplastic anemia can occur during or immediately after a fairly long period of exposure to TNT. Several cases were preceded by an episode of toxic jaundice or hepatitis (45).

As to the size of RBC, monocytic and megalocytic cells were found in patients intoxicated with TNT (34).

Hemolytic anemia was also reported especially in workers deficient in the enzyme, glucose-6-phosphate dehydrogenase [G6PD] (39, 40).

White Blood Cells (WBC): An early reaction to TNT exposure is an increase in the large mononuclear leukocyte count which usually precedes any clinical symptoms from intoxication with this compound. It can, therefore, be used as a test for the differential diagnosis of TNT intoxication. This is usually followed by a decrease in the number of polymorphonuclear leucocytes and an increase in the lymphocytes (34, 47).

Blood Platelets: The number of blood platelets is decreased in certain cases of TNT intoxication. The low platelet count together with the increased fragility of capillaries leads to toxic purpura (34).

Bone Marrow: Hyperplasia of the bone marrow is the first reaction of the hemopoietic tissues to TNT toxicity (34). This reaction was ascribed to the increased oxygen demand caused by the altered hemoglobin, as well as by the products of blood destruction (36). However, further intoxication with TNT depresses the blood-forming tissues, and the bone marrow becomes hypocellular (34).

c. Cardiotoxicity

Because of the overwhelming toxic effects of TNT on the liver and blood, less attention has been paid to the cardiotoxicity. Soboleva (49) observed abnormalities including systolic murmurs, hypotension, reduced atrial and atrioventricular conduction rates, systolic prolongation and myocardial dystrophy in a group of 150 TNT workers (38 men, 112 women, age 40 and under in 90% of cases) 81% of whom had been exposed for more than 5 years.

d. Vasotoxicity

An increased vascular permeability, especially for proteins, was observed in workers suffering from TNT poisoning (50). This increase in permeability is in both directions, viz., from the vessel into tissues and vice versa.

e. Toxicity to Pancreas

Functional disorders of the exocrine activity of the pancreas, in the form of dyspancreatism of enzymes of the pancreatic juice and diastase deviation after double load with glucose, were observed in patients with TNT poisoning (51).

f. Dermatotoxicity

TNT-caused dermatitis usually starts after 5 or more days of exposure and is often found in points of friction. It commonly starts between the fingers the free edge of the palm. Usually skin lesions heal when exposure to TNT is stopped. Dermatitis is more prevalent where TNT levels are usually higher, as in melting and pouring TNT into shells. It becomes more serious during hot and humid weather (28, 53, 54).

g. Oculotoxicity

Chronic TNT intoxication resulted in the cataract formation in several workers (57, 58, 59, 60, 61). This cataractogenic effect may be due to a direct effect on the lens (63, 64), and is often the first and only sign of the presence of TNT in the organism (66). The development of TNT cataract is

gradual and usually takes several years; 2 to 3 years in man, and up to 6 years in women, significantly exposed to TNT (66). Some investigators believe that the features of this cataract are characteristic to TNT. When contact with TNT was stopped the development of cataract was retarded and even partial resolution of the opacities occurred. For further details, please refer to section (G) in chapter III.

h. Teeth and Oral Cavity Toxicity

Carious and non-carious tooth injury as well as peridontal and oral cavity mucous membrane diseases were observed (69).

i. Neurotoxicity

Workers handling TNT showed a significant decrease in the chronaxy of the flexors and extensors of the hand (70, 71) as well as definite prolongation of the optical, vestibular and motor chronaxy (18). A disorder in the thermoregulating reactions to heat and cold was also observed in 50% of workers (18).

j. Nephrotoxicity

A significant rise in the glomerular filtration rate and partial sodium retention was observed in TNT-exposed workers (75). Incidents of porphyrinurea were also reported among TNT workers (77). In mild cases of TNT poisoning, urgency, frequent micturition and lumbar pain may be the only complaints (76).

k. Biochemical Changes

A significant decrease in plasma proteins in over 70%, and a significant increase in the plasma bilirubin in 20% of persons exposed to TNT were observed. No significant change in the levulose tolerance test (78), nor in the SGOT, SGPT, or SDH (79) were observed in one study. However, an increase in SGOT and lactic dehydrogenase (LDH) was observed by Morton and Ranadive (177) in workers exposed to levels of TNT as low as 0.6 mg/m^3.

B. CLINICAL MANIFESTATIONS OF TNT POISONING

a. Signs and Symptoms

Symptoms of TNT poisoning may be mild and due mainly to irritation of skin and respiratory passages, or severe and due to absorption of sufficient amounts of TNT to produce toxic symptoms.

i. Mild Irritative Symptoms: These include:

- Nasal discomfort, sneezing, epistaxis, coryza, sore throat and dry cough (26).
- Dermatitis and erythema. Desquamation and complete exfoliation might follow (80).
- Gastritis, nausea, vomiting, anorexia, constipation and epigastric pain (27).

ii. Severe Toxic Symptoms: These are due to the absorption of sufficient amounts of TNT and are manifested by:

- Cyanosis, first manifested in lips, tongue, ears, fingertips and mucous membranes (6,81).

- Toxic jaundice, which indicates severe liver damage and is responsible for 30% of the fatalities from TNT poisoning (82).

- Aplastic anemia; this is usually fatal and is sometimes preceded by jaundice (83,84).

- Cataract; this is considered by some investigators as the first and only clinical sign of intoxication (85).

- Menstrual disorders and dysuria (86).

- Neurological manifestations in the form of neurasthenia, nystagmus, irregularity of tendon reflexes and adiadochokinesia were also manifested. Vagotonia that resulted in hyperhidrosis of feet and hands, acrocyanosis and bradycardia were reported (87).

b. Findings on Physical Examination:

- Yellow discoloration of the skin, nails and hair (88).

- Cyanosis, especially manifested in lips and sclera (89).

- Dermatitis (53,55,56)

- Epigastric pain, tenderness and spasm in the epigastrium (27).

- Enlarged liver, that is distinctly palpable below the costal margin.

- Decrease in the diastolic and mean arterial pressure, an increase in stroke and minute volume; bradycardia, a decrease in the amplitide of the QRS complex and flattening of the T wave (87).

- Decrease in the amplitude of the bioelectric activity in the brain (87).

c. Laboratory Findings

- Urine of patients exposed to TNT is usually darker in color than normal, and in most of the cases gives a positive Webster's test (206).

- Blood shows normochromic anemia, leukopenia, lymphocytosis and thrombocytopenia (87).

C. TREATMENT OF TNT TOXICITY

Since there is no specific antidote for TNT poisoning, the following procedures are necessary:

a. Immediate removal from all contact with TNT, changing of any contaminated clothes and thorough washing of the skin with soap and water (2).

b. High carbohydrate diet with plenty of fresh food and vegetables to support the liver. Relapses of jaundice are common and patients must be watched for an extended period of time (84).

c. Dermatitis can be treated by the application of mild wet dressings such as boric acid solution or calamine lotion. Should lesions become infected antibiotic ointments are recommended (53,94).

d. Though prognosis is poor, frequent transfusion of fresh blood and high vitamin C and B complex intake are necessary measures to combat aplastic anemia. Extreme care should be taken to guard against any infection or even injudicious doses of bone-marrow depressant drugs (34,42).

e. Cyanosis can be treated with oxygen and carbon dioxide inhalation.

D. HUMAN FATALITIES

The greatest number of human fatalities occur in the melting, pouring, pressing, boring and planing of the charges of shells. Death is mainly due to jaundice, aplastic anemia or both. Both dust and fumes of TNT gain access to the body via the bronchial tree, and TNT can be also absorbed through the skin. The first fatality attributed to TNT poisoning was in 1915 and it was due to jaundice. By the end of that year, no less than 50 other cases of toxic jaundice that ended fatally were reported among TNT exposed workers (97). In 1917, 95 other cases were mentioned, of which 28 ended in death (95). In the U.S.A., 475 fatal cases were reported during World War I. In another estimate 580 died from TNT poisoning in the U.S.A. in the period of 1914 to 1918 (159). In the same period 90 workers died in England. In Germany, however, only 20 TNT workers developed liver atrophy and died (98).

Strict hygienic measures during World War II resulted in a dramatic decrease in the number of fatalities due to TNT poisoning. Thus, only 22 patients died in the U.S.A.; 8 of them died of toxic hepatitis, 13 of aplastic anemia and one from both. Sporadic cases were reported after World War II, and also other causes of death, such as thrombocytopenic purpura were mentioned (34).

Postmortem Findings:

Microscopic examination revealed fatty degeneration of the liver and kidney, and marked cellular infiltration of the connective tissue stroma in lungs (105,106,107).

E. SUSCEPTIBILITY TO TNT POISONING

Women are more susceptible to TNT poisoning than are men; the immature workers of either sex are more susceptible than the mature ones (95) and black people are much less susceptible to TNT toxicity than whites (53).

Morton et al. (109) found a significant increase in SGOT and LDH in patients exposed to as low a level of TNT as 0.6 mg/m^3. The present U.S. Occupational Safety and Health Administration Standard for TNT in the atmosphere is 1.5 mg/m^3 (8-hour time weighted average).

3. TOXICOLOGIC INVESTIGATIONS IN ANIMALS

Due to the fact that most of the animal experiments were carried out during World Wars I and II, the design of the experiments, the number of animals used, as well as the exact dose of TNT given via various routes, would not meet present protocols. For instance, some authors used only one animal for testing the toxicity of TNT when given orally; after several days when no ill-effects were exhibited, the same animal would be used for percutaneous administration of TNT. However, animal toxicity is discussed under the following topics:

A. Toxic dose levels These are summarized in Table IV.1.

B. Species sensitivity and tolerance

Cats are very sensitive to TNT, whereas, monkeys, rabbits and rats showed less sensitivity to the compound. This difference was suggested to be due, in part, to the different fate of TNT in the organism (66). Tolerance is not acquired by experimental animals to TNT (112).

C. Toxicological differences between TNT isomers

While authors believe that β- and γ- isomeric trinitrotoluenes are of the same qualitative and quantitative toxicity as TNT (66, 110), others claim that the latter is less toxic than the β- and γ-isomers (113). However, the presence of β- and γ- compounds does not affect its efficacy as an explosive.

D. Acute Toxicity

a. Mice: The oral LD_{50} for male and female animals were 1,014 ± 52 mg/kg, and 1,009 ± 54 mg/kg, respectively (164).

b. Rats: The acute oral LD_{50} of TNT administered as 4.12% solutions in peanut oil was found to be 1,010 ± 41 and 820 ± 32 mg/kg in male and female rats, respectively(153). Subcutaneous injection of 0.5 to 1.0 g/kg of TNT in arachis oil to Wistar rats resulted in death of a "proportion" of the animals within 48 hours (32). A suspension of TNT (2% in acacia mucilage) given orally or subcutaneously to male albino rats every 2 days for 18 days, resulted in an increase in the porphyrin excretion only in doses higher than 400 mg/kg (110).

c. Guinea Pigs: Oral administration of TNT to guinea pigs in doses of 0.2 to 0.5 g daily or every other day for 3 to 8 days resulted in the appearance of severe anemia of the hypochromic type with an increase in the reticulocyte count, considerable leukocytosis and reduced platelet count(121).

Moderate dermal sensitivity was noted after topical application of a 4.12% solution of TNT in peanut oil, according to the technique of the Magnusson and Kligman "maximization test".

d. Rabbits: TNT brought about a decrease of 50% in the total plasma proteins, mainly in the albumin fraction. Doses of 0.65 g/kg of TNT given subcutaneously induced hypochromic anemia with acute leuko- and thrombocytopenia.

Primary skin and eye irritation was considered to be mild following tests with a 50% paste of TNT in peanut oil, according to a modified Draize method. A red pigment developed under skin patches and around the eyes of all treated rabbits within 24 hours (114).

e. Cats: TNT injected intraperitoneally to cats in doses of 0.10 to 0.15 mg/kg was lethal within 3.5 to 5.5 hours. Doses of 0.04 g/kg and over caused phagocytosis of erythrocytes, hemosiderosis of Kupffer's cells and moderate fatty degeneration of the liver.

f. Dogs: TNT given intravenously to dogs in a dose of 1.5 mg/kg decreased the chronaxy of the hind legs 3 to 6 hours after the injection.

E. CHRONIC TOXICITY

a. Monkeys: Rhesus monkeys orally administered TNT in doses of 1.0, 0.1 or 0.02 mg/kg b.w./day for 13 weeks experienced gagging and vomiting, and reduced weight gains, but no abnormalities were detected in blood chemistry, urine, behavior, appearance, motor activity or liver function (BSP clearance). Ophthalmological abnormalities were not noted. The highest dose produced increased liver cord cell hemisiderin deposits as compared with controls. Bone marrow examination revealed absence of observable normal megakaryocytes, but thrombocytopenia could not be confirmed (224).

b. Rats: TNT was given daily (0.15 g/kg) mixed with food of 3 basic diets (protein-, carbohydrate- and fat-rich diet). Only those fed on a fat-rich diet showed, within the first two weeks, signs of weakness, anemia and liver lesions in the form of fatty infiltration and acute necrosis of parenchymal cells. An erythroblastic hyperplasia of the bone marrow and siderosis of the spleen was also detected. Death occurred within 4 to 6 weeks (32). A biphasic response in excitability of the neuromuscular junction (reduction followed by increase) was observed when rats were given 30 mg/kg/day TNT orally for 60 days (163). TNT decreased the catecholamine levels in the heart and brain of rats two months after the beginning of poisoning. The serotonin level was also decreased and the monoamine oxidase activity was increased in the liver and brain of rats given 100 mg/kg/day of TNT orally for 3 to 30 days (191). Various methods to determine the effect of TNT on liver function in albino rats were also mentioned (42).

c. Guinea Pigs: Administered by inhalation, TNT produced, at the beginning an increase in the number of the erythrocytes; anisocytosis, poikilocytosis and polycythemia were observed (85). Given orally, it produced hyperchromic anemia, moderate leukocytosis and moderate reduction in the number of blood platelets (124). The chronic effects of TNT on the nervous system of guinea pigs consist of microfocal destruction of white matter in the brain, and the vessels were enlarged and plethoric (74).

d. Rabbits: The effect of chronic intoxication with TNT on the blood picture was studied (76,121) . A decrease in platelet count was ascertained,

but changes in prothrombin, coagulation, or clot retraction time were not observed. Methemoglobinemia (172) and an increase in γ-globulin (111) were also observed. In a total dose of 0.08 g given over 4 weeks, TNT increased the urinary coproporphyrin level(176). A progressive decrease in the total quantity of plasma proteins (9)as well as various biochemical changes(136-138)were also reported.

e. Cats: A total dose of 9.5 g of TNT given to a 3.8 kg cat by rubbing on the skin caused death within 18 days (173).

f. Dogs: Beagles administered 0.02-1.0 mg/kg b.w./day of TNT orally for 90 days developed no toxic manifestations other than temporary episodes of vomiting during the first 14 days (223). TNT given orally in doses of 5 to 33 mg/kg caused cyanosis, salivation, diarrhea, incoordination, icterus and anemia (112). In another experiment TNT was given orally, 6 days/week for 4 weeks in doses of 5, 15, 25 and 100 mg/kg b.w. During the first 7 to 10 days dogs appeared extremely sick, ataxic and manifested incoordination and nystagmus. By the end of the second week, however, animals showed marked improvement and returned to normal(142). Oral administration of 50 mg/kg day of TNT to a dog for 12 weeks did not prove fatal (179). Some inhalational studies with negative results were also reported (129). When injected subcutaneously every other day, in a dose of 20 to 50 mg/kg/day for 3 months, TNT caused depression of bile formation and excretion.

g. Microscopic changes in experimental lesions: Microscopic changes that were observed in various animal species are summarized in Table IV.3.

4. CARCINOGENICITY, MUTAGENICITY AND TERATOGENICITY OF TNT

To date, no carcinogenic effects from TNT intoxication, nor teratogenic studies have been reported. Schepers (149)could not find any pulmonary lesions including lung cancer in guinea pigs, rats and mice that were exposed to TNT. In concentrations of 0.5 to 10 μg/ml, TNT, studied by the Ames assay, was found to be a frameshift mutagen (150). Chromasomal and chromatid abnormalities were noted in bone marrow cells of rats after 6 months of topically applied 30% TNT 5 times a week (222).

5. RELATIONSHIP BETWEEN DIETARY FACTORS AND TNT POISONING

Experimental investigations in animals revealed that dogs fed a meat diet are more resistant to TNT poisoning than dogs which are fed white bread and milk (112). The addition of milk, on the other hand, to the diet of dogs that were given 50 mg/kg TNT orally, alleviated only slightly the toxic effects produced by TNT. A fat-rich diet increased the toxicity of TNT in white rats.

The effect of the addition of vitamin C to the diet of rats, guinea pigs, rabbits and cats was studied and it was found that only in the case of cats that ascorbic acid appeared to have a slightly favorable influence on the course of TNT intoxication. The degenerative changes induced by TNT in the liver were inhibited by giving methionine, liver extract, B complex and nicotinamide to rabbits poisoned by TNT.

Observations in humans included a favorable effect of Vitamin C and a deleterious effect of alcohol on the course of TNT toxicity.

6. ABSORPTION, DISTRIBUTION, BIOTRANSFORMATION, EXCRETION AND BIODEGRADATION OF TNT

Absorption and distribution: TNT gains access to the body through lungs, gastrointestinal tract and/or the skin, which is the chief route of absorption. TNT is distributed to various organs and tissues as shown in Table VIII.1.

Biotransformation of TNT: Various *in vivo* and *in vitro* experiments were conducted to study the biotransformation of TNT. It was found that TNT can be reduced to give mainly 2,6-dinitro-4-aminotoluene and 2,4-dinitro-6-aminotoluene. The sequences of events and the various intermediates in the reduction of TNT are shown in Figure VII.1. On the other hand, TNT undergoes oxidation and various hypothetical routes are illustrated in Figure VII.2. TNT is mainly excreted in the urine.

Microbial Degradation of TNT: Various organisms are able to biodegrade TNT. *E. coli* can reduce at least one nitro-group of TNT to its respective amine (172, 174). The enzymatic process requires NADH. Pseudomads isolated from mud and water can metabolize TNT to isomers of dimethyltetranitrobenzine, and dinitrohydroxylaminotoluene and nitrodiaminotoluene (170,171). *Pseudomonas* I and II, isolated from rat feces and raw sewage, aerobically degrade TNT and incorporates it into the Krebs cycle (170).

7. EPIDEMIOLOGY

Several fatalities that occurred due to TNT exposure are enumerated in Chapter VIII. Cases of mild intoxication, medical transfers of employees to jobs not involving TNT exposure, due to the development of dermatitis or systemic intoxication, and fatalities were summarized in Tables VIII.1 and VIII.2.

Various health hazards, safety measures and standards for TNT are summarized in Chapter IX. The detection and estimation of TNT in air and in various biological fluids are enumerated in Chapter X.

XII. CONCLUDING REMARKS AND RECOMMENDATIONS

A large amount of information on the adverse health effects of TNT has already been compiled, as is evident in the preceding discussion, in this review. Unfortunately, much of this was gathered from direct observation of the poisoning of industrially exposed humans, before being examined in suitable animal models. However, there do remain areas where more information may be gathered in order to develop informed regulations that will protect the TNT worker. These are enumerated as follows:

1. To date cancer is not claimed to have been caused by TNT exposure to humans or other animals. However, it may require 20 years or more for a latent tumor to become detectable in humans. More than 30 years has passed since a large number of workers were exposed in the production of TNT for use in World War II. Therefore, it seems the ideal time to institute a retrospective cancer study of these workers.

2. Reproductive effects have not been sought in human epidemiological studies or experiments with animal models. Such a study might be initiated to ascertain if the number of birth defects are increased, or fertility and other changes are evident in workers recently exposed to TNT.

3. Item one may be further supported by appropriate carcinogenesis bioassays in experimental animals. Reproductive and teratogenic effects may be examined using animal models rather than an epidemiological survey.

4. TNT is known to produce hemodynamic effects, liver disease, and possibly cataracts. To protect workers the U.S. Occupational Safety and Health Administration has adopted the threshold limit value of 1.5 mg/m^3 (8 hour time-weighted-average) as the standard for atmospheric exposure to TNT. However, several authors (100, 176, 177) have questioned the adequacy of this level. Therefore, inhalation experiments in which sensitive animals are exposed for 8 hours/day to atmospheric levels below 1.5 mg/m^3 may be appropriate to determine if any signs of toxicity are produced. Cataract development studies should be considered.

5. To date, there is no single and specific test to detect the early symptomless stages of TNT toxicity. Therefore, a test for the detection of TNT in biological fluids, that is sensitive and specific, should be developed.

6. Although the content of impurities in military grade TNT is only about 0.2%, the possibility exists that these impurities, e.g. isomers other than α-TNT as well as other products formed in the nitration of toluene, may contribute to or enhance the toxicity and/or mutagenicity of TNT. For these reasons, the oral LD_{50}, skin sensitization, eye and skin irritation tests in appropriate animal models, as well as the Ames test, should be investigated in order to derive toxicological data for TNT isomers of importance to personnel who may be exposed during its manufacture and use.

7. Since the toxicity of TNT may, in part, be attributed to one or more of its metabolites, especially 2,4-dinitro-6-aminotoluene and 2,5-dinitro-4-aminotoluene, an assessment of the relative toxicity of these to TNT in various animal species is warranted.

8. Certain medications are known to cause intrahepatic cholestasis, hepatocellular necrosis or miscellaneous histopathological lesions in the liver. Investigations of possible interactions of these compounds with TNT might be of value. In addition, persons using these drugs should be aware of the risk of serious liver damage with TNT exposure. Examples of these drugs include phenothiazine derivatives, some antibiotics, diuretics, oral contraceptives and other steroid hormones. A more complete list may be found at the end of this chapter.

9. In the same consideration as item 8, persons taking drugs which are toxic to or depress the bone marrow, should be aware that TNT is itself a bone marrow depressant. A list of these medications, also at the end of this chapter, includes certain antimetabolites, antineoplastic compounds, antihistamines, anticonvulsants, benzene, toluene and chloramphenicol.

10. Since TNT has been reported to induce hemolytic anemia in persons deficient in glucose-6-phosphate dehydrogenase (G6PD), the implications of this finding as a pre-employment examination test for TNT workers should be further investigated.

11. Specific toxicological studies that have not been performed on TNT, and the gaps that should be filled are indicated in Table XII.1., which follows.

TABLE XI-1
GAPS IN TOXICOLOGICAL DATA ON TNT

Acute dermal LD_{50}	X
Metabolism[a]	X
Mutagenesis	(in progress)
2-year feeding[b]	X
180-day feeding	X
Reproduction	X
Teratology	X

a. Metabolism includes absorption, distribution, excretion and pharmacokinetics, using radio-labeled material; and includes the identification and possible isolation of any metabolites.

b. This includes a carcinogenicity evaluation.

X = Study has not been performed

Hepatotoxic Substances

1. Alpha-methyldopa (Aldomet)
2. Carbarsone (Amebarsone)
3. Ectylurea (Cytran, Levanil, Nostyn).
4. Methimazole (Tapazole)
5. Phenothiazine derivatives:
   a. Chloropromazine (Thorazine)
   b. Promazine (Sparine)
   c. Perphenazine (Trilafon)
   d. Prochlorperazine (Compazine)
   e. Mepazine (Pacatol)
   f. Thioridazine (Mellaril)
   g. Trifluoperazine (Stelazine)
6. Chlorothiazide (Diuril)
7. Thiouracil
8. Sulfonylurea derivatives
   a. Chlorpropamide (Diabinase)
   b. Tolbutamide (Orinase)
9. Erythromycin estolate (Ilosone)
10. Sodium oxacillin (Prostaphlin, Resistopen)
11. Nitrofurantoin (Furadantin)
12. Imipramine (Tofranil)
13. Acetohexamide (Dymelor)
14. Amodiaquine (Camoquin)
15. Chlordiazepoxide (Librium)
16. Ethionamide (Trecator)
17. Isoniazid
18. a. Halothane (Fluothane)
    b. Methoxyflurane (Penthrane)
19. Monoamine Oxidase Inhibitors
    a. Isocarboxazid (Morplan)
    b. Niolamide (Niamid)
    c. Phenelzine (Nardil)
    d. Tranulcyclopromine (Parnate)
20. Phenylbutazone (Butazolidin)
21. Probenecid (Benemid)
22. Sulphonamides:
    a. Sulphamethoxazole (Gantanol)
    b. Sulphasoxazole (Gantrisin)
    c. Sulphamethoxypyridazine (Kynex, Midicel)
23. Amphotericin B
24. Chlorambucil (Leukeran)
25. Chloramphenicol (Chloromycetin)
26. Phenyton Sodium (Dilantin)
27. Gold salts
28. Male fern
29. Mercaptopurine (Purinethol)
30. Metahexamidel (Euglycin, Melanex)
31. Methsuxamide (Celontin)
32. Nicotinic acid
33. Novobiocin (Albamycin, Cathomycin)
34. Para-amino salicylic acid (PAS)
35. Penicillin
36. Phenacemide (Phenurone)

37. Phenacetin
38. Phenindione (Hedulin)
39. Triacetyloleandomycin
40. Trimethadione (Tridione)
41. Uracil Mustard
42. Gall bladder x-ray dyes
    a. Bunamiodyl (Orabilex)
    b. Iopanoic acid (Telepaque)
43. Adrenal and Adrenocorticotrophic steroids
44. Trichloroethylene (Trilene)
45. Amethopterin (Methotrexate)
46. Ethyl carbamate (Urethan)
47. Tannic acid (Chysodrast)
48. Methyltestosterone
49. Norethandrolone (Nilevar)
50. Methandrostenolone (Dianabol)
51. Oral Contraceptives:
    a. Norethynodrel with Mestranel (Enovid)
    b. Megestral acetate (Volidan)
    c. Lynestrenol (Lyndiol)

Substances Toxic to Bone Marrow

1. Ionizing radiation
2. Alkylating agents
    a. Nitrogen Mustard (Mustargen)
    b. Triethylene melamin (TEM)
    c. Busulfan (Myleran)
3. Ethyl carbamate (Urethane)
4. Benzene
5. Antimetabolites
    a. Antipurines
    b. Antifolic acid
6. Chloramphenicol (Chloromycetin)
7. Anticonvulsants
    a. Mephenytoin (Mesantoin)
    b. Butalbarbital (Butisol)
    c. Phenytoin (Dilantin)
    d. Ethosuximide (Zarontin)
8. Antithyroid drugs
    a. Imidazole (Carbimazole)
    b. Methimazole (Tapazole)
    c. Potasium perchlorate
    d. Thiocyanates
9. Antihistamines
    a. Tripelennamine (Pyribenzamine)
    b. Diphenhydramine (Benadryl)

10. Glue
    a. Toluene
    b. Benzene

11. Certain Insecticides

12. Phenylbutazone (Butazolidin)

13. Acetazolamide (Diamox)

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APPENDIX
INFORMATION SOURCES EXAMINED

Computer Searchable Data Bases

1. National Technical Information Services - covering 1964 to present (searched on 4/4/77).

2. Toxline/Toxback (searched on 3/29/77)

3. Chemical Condensates - covering 1972 to present (searched on 4/1/77)

4. BIOSIS Previews - covering 1972 to present (searched on 4/8/77)

5. ISI SCISEARCH - covering 1974 to present (searched on 4/8/77)

6. CANCERLINE (searched on 5/5/77)

7. NIOSH Technical Information Center file - (received on 5/15/77)

8. Defence Documentation Center - (received on 5/15/77)

Hard Bound Secondary References

1. Chemical Abstracts - V. 1 (1907) - V. 83 (1975).

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4. Engineering Index - (covering 1940 through 1977, issue #3).

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Organizations Contacted

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2. U. S. Army Environmental Hygiene Agency, Edgewood Arsenal, Md.

3. Naval Environmental Hygiene Center, Cincinnati, Ohio.

4. U.S. Navy Medical R&D Command, National Naval Medical Center, Bethesda, Md.

Persons Contacted

1. Crawl, Mr. James
   U.S. Naval Environmental Hygiene Center
   Cincinnati, Ohio

2. Doptis, Lt. Commander Leigh
   Naval Medical R&D Command
   National Naval Medical Center
   Bethesda, Maryland 20014
   (301) 295-1131 or (301) 395-1028

3. Hathaway, Lt. Col. James
   U.S. Army Environmental Hygiene Agency
   Edgewood Arsenal, Maryland
   (301) 671-2304

4. Miller, Mr. Lester
   Chemical Systems Lab.
   U.S. Army Environmental Hygiene Agency
   Edgewood Arsenal, Maryland
   (301) 671-3557

5. Owens, Mr. Ed
   Chemical Systems Lab.
   U.S. Army Environmental Hygiene Agency
   Edgewood Arsenal, Maryland
   (301) 671-2129

6. Taft, Mr. Richard
NIOSH
DTS, COSHI, IRAS, C-19
4676 Columbia Parkway
Cincinnati, Ohio 45226